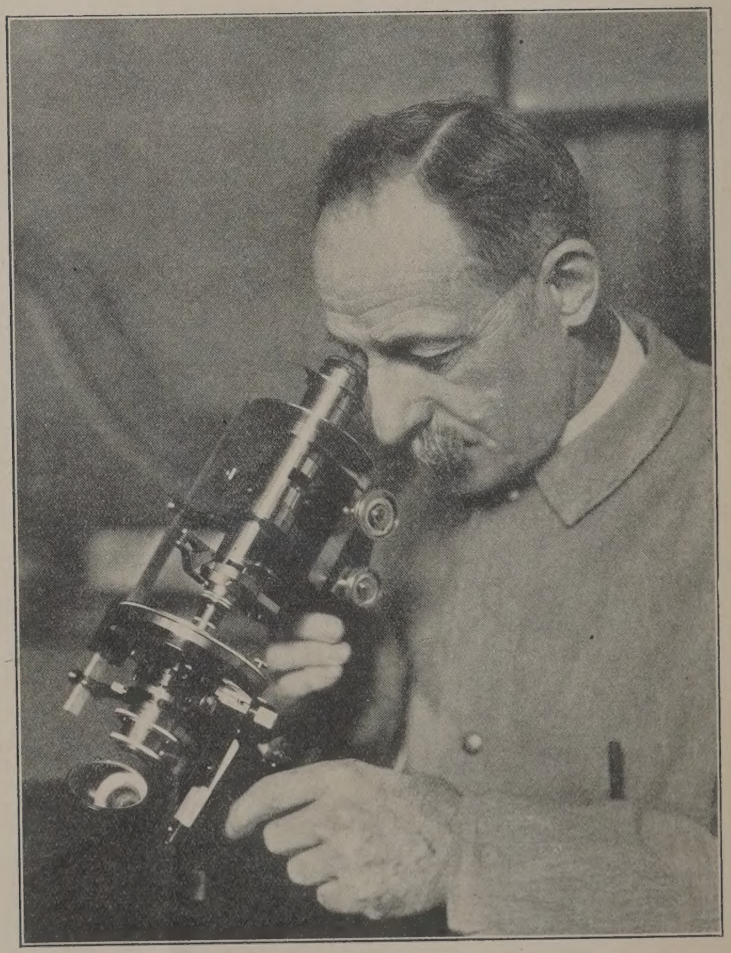
Winches Farm,
Hatfield Road,
St. Albans, Herts.



Nathan Augustus Cobb, June 30, 1859–June 4, 1932

The Journal of Parasitology

Volume XIX SEPTEMBER, 1932 Number 1

NATHAN AUGUSTUS COBB

MAURICE C. HALL

Dr. N. A. Cobb was born June 30, 1859, in Spencer, Mass. Both of his parents were of old New England families. As a boy he showed an excessive interest in his school work, and at one time had to be taken out of school and put to work because of a growing habit of remaining awake at night to think over his studies. He was accustomed to labor and divided his time between school and jobs of various sorts, among other things teaching for two years in the public schools in Spencer. While he never finished his formal secondary school education, he made up his work by reading and entered the Worcester Polytechnic Institute, receiving his B.Sc. in 1881. Soon after graduation he married Alice Vara Procter who was also of old New England stock. For the next six years he taught chemistry and natural science in Williston Seminary, saving money at this time for the graduate work in Germany which he felt was necessary. He then went to Jena where he worked under Haeckel, Hertwig, Lang and Stahl, receiving his Ph.D. in 1888.

Having concluded the business of obtaining a formal education, Dr. Cobb began a series of wanderings which kept him engaged in a variegated range of studies over a large part of the world. His doctorate thesis was a study of nematodes, and in his subsequent work a study of nematodes, or nemas as he preferred to call them, was carried on either as his major work or as an associated line. While he worked from time to time with the parasitic nematodes, his principal interest was with the free-living or plant-parasitic forms, and he was one of the very few men who in his life time knew very much about these generally neglected and little known worms. He was a striking combination of zoologist and technologist, a man who combined the technical and mechanical ability of the Yankee with the scientific quality of the German school. Extremely capable and ingenious with tools, he could construct a camera or a microscope, and knew more about optics than do most physicists. His laboratory was a show place and visitors accustomed to routine scientific methods and customary equipment were impressed and more or less bewildered with the careful

procedures and the elaborate and efficient apparatus in Dr. Cobb's laboratory. All his microscopes used in fine work were established on deep-seated supports to prevent vibration, room light was excluded and light was obtained from canvas "clouds" with special coating and adjustable to the position of the sun. Microscopes for less fine work were arranged on rotating tables so that things could be brought around to the position of the worker instead of the worker moving to the things needed, and around the laboratory were the differentiators, thermolethes and other devices of which he was the inventor.

His first move from Jena was to the Naples Zoological Station as the appointee of the British Association for the Advancement of Science, this appointment being an unusual compliment in the selection of an American for work at the expense of the British Association. After a year at Naples, Dr. Cobb wished to visit Australia, and as his meagre funds were exhausted he borrowed money from a friend in his Massachusetts home and sailed with his family to Sydney. He had no position in sight, but he had letters of introduction—and his ability and confidence. During his first month in Sydney he spent his mornings in presenting his letters of introduction to persons who might secure a position for him, and his afternoons in microscopical investigations carried out at his residence. At the end of a month he obtained a position with a commercial house, one of his duties being to write advertisements. He carried out this work with his characteristic thoroughness and initiative. A consignment of American soap and American watches was to be advertised. He cut out pieces from bars of American soaps and English soaps, and carried out tests showing the superiority of one of the American soaps. He wrote the story of the American watch and undertook to publish a full-page advertisement, with illustrations, in one of the Sydney papers. No illustrated advertisements had ever been published in the Sydney papers and the first paper approached on the subject refused to indulge in this novelty. However, the Sydney Telegraph agreed to publish the advertisement and did so at a price which shocked Dr. Cobb's employer. Simultaneously with the publication of the advertisement, Dr. Cobb had a jeweler's window filled with the watches, showing some of them running immersed in water. This exhibit drew great crowds and the venture was a financial success for his employer.

In 1890, Professor Haswell of the University of Sydney left on a year's leave of absence, and Dr. Cobb was appointed *locum tenens* in his position. He helped to organize the Department of Agriculture of New South Wales and from 1891 to 1898 he was pathologist of that Department. For the last year of this period he was also manager of the Wagga Experiment Farm. From 1898 to 1901 he was Agricul-

tural Commissioner to the United States and Europe, carrying out extensive investigations on wheat and other things, and then returning to his position as pathologist from 1901 to 1904. During this period in Australia he worked on rust-resistant wheats, continued his work on free-living and plant-parasitic nematodes, of which he described a number, and on parasitic nematodes of animals, and published some excellent works on parasites of livestock, one of which, the very important common sheep stomach worm, he placed in the new genus Haemonchus. In Australia he became acquainted with Mr. W. E. Chambers, an excellent artist, engraver, and technician, and the latter became associated with Dr. Cobb at that time and followed him in his subsequent moves up to the time of the death of Mr. Chambers in Washington in 1920. During the time of their association, Dr. Cobb's papers were illustrated with engravings made by Mr. Chambers or from drawings by him, and these illustrations are wonderfully clear and detailed.

From Australia Dr. Cobb went to Hawaii, and early in 1905 organized the Division of Physiology and Pathology of the Hawaiian Sugar Planters' Experiment Station. He was director of this Division until 1907. Here he worked on nemic and fungus diseases of sugar cane, and continued his investigations of free-living nemas.

In 1907 he went to Washington, D. C., as Agricultural Technologist in the U. S. Department of Agriculture. His first major occupation was the standardization of cotton types, a technological procedure for which he had unusual equipment, and he invented a form of vacuum storage for these types. For some years he was Acting Assistant Chief of the Bureau of Plant Industry, but at all times he continued his investigations on nemas, and under the Reclassification Act he was finally given the title of Principal Nematologist.

A survey of his published papers discloses evidence of his originality and ingenuity. He brought to his study of nemas a sound knowledge of chemistry and physics, as well as of zoology and technology. He used the polariscope to study the birefringent granules of the intestinal cells, and chemical reagents to investigate the nature of the nemic structures. He constantly raised questions as to how and why in connection with everything he saw, and while he was consistently scientific in proposing his explanations in tentative form, he was also immensely ingenious in proposing and examining the possible explanations.

Dr. Cobb was known to a wide circle of scientists throughout the world. He was a member of numerous American and foreign scientific societies, and was a president of the American Microscopical Society, the American Society of Parasitologists, the Washington

Academy of Science, and the Helminthological Society of Washington. In the informal and intimate meetings of the Helminthological Society, he will be missed acutely, not only because of the interesting facts that he brought to its attention and the stimulating questions he proposed, but because of his personal charm. He combined frankness with courtesy, and an unshaken dignity with a keen sense of humor. He had unusual histrionic ability, and at the dinners of the society he displayed an actor's qualifications in "reading his lines" with the precision and polish appropriate to the occasion. Not infrequently he wrote delightful verse for these dinners, and at the last meeting of the society before his death he read some charming nonsense about his nemas, written on the interurban on his way into Washington from his home in Falls Church, Va. The society will long remember a dinner at which he presented a discussion of the collection of "holes" he claimed to have made, the presentation being accompanied by an exhibit of these holes, including the oldest extant geological specimen from which all the surrounding rock had been carefully chipped to leave nothing but this most ancient of cavities.

On June 4, 1932, Dr. Cobb was in Baltimore, Md., having his annual physical examination. From early life he had had some heart irregularities, probably the sequelae of an attack of scarlet fever when he was about two years old, and this condition necessitated these examinations and some treatment. At 9:00 P. M. he was in bed reading and apparently feeling well and comfortable. When the nurse entered the room a short time afterward he was dead. The passing of this able scientist and dear friend is an occasion for regret and sorrow, and we who knew him shall miss him greatly. But these regrets and sorrows are for ourselves, not for our friend, for there is no better alternative in leaving life than to go quickly and while physically fit and mentally unimpaired at the end of a long life of constructive achievement and appreciative friendships. To the wife who has followed him throughout his wanderings and shared his hardships, and to the children who have loved and honored him, we extend our sympathy. The tall figure has gone from us, and we shall see no more the intellectual face with its crescentic monocle and the smile for which we looked, but the memory of the man is pleasant and abiding. In no uncertain sense he remains with us and will long remain with us.

THE LIFE-CYCLE OF MONIEZIA EXPANSA

R. Daubney
Division of Veterinary Research, Kenya Colony

Recent publications on the life-history of the Anoplocephalid tapeworms of sheep (Konsuloff, 1929, Mönnig, 1929, Sinitsin, 1931, Scott & Honess, 1931) encourage the writer in the belief that a brief survey of the literature, together with records of certain experiments carried out in England in 1923, may be of interest to workers engaged in the study of this problem. The results of the experiments have never been published, although they were communicated to the Congress of the National Veterinary Medical Association held at York, England, in August 1923 in the course of a discussinon on a paper by the writer (Daubney, 1923).

Most of the earlier literature relating to studies on the life-history of Moniezia of sheep has been reviewed by Flattely (1922), who pointed out that there are two distinct viewpoints from which suggestions have been made: the first that the services of an intermediate host (probably an invertebrate) are required, and the second that the adult tapeworms may develop directly from the eggs without the intervention of any intermediate host. The early age at which lambs are known to contract infestation (according to Spinola one month, Cooper Curtice two months, Flattely four to six weeks, Daubney two months), has led to further suggestions that infestation may be prenatal, or that alternatively the lamb may acquire its infestation from the milk, or from the exterior of the udder of the ewe. We have thus to deal with four distinct possibilities, direct or indirect transmission, ante or post-natal infestation, and in the latter case it is necessary to consider also whether infestation is acquired from the milk or udder of the ewe or from some extraneous source.

DIRECT TRANSMISSION

Cooper Curtice claimed to have obtained infestation in stabled lambs kept under such conditions "that none of the invertebrates which are usually suggested as being the intermediary bearers were present" (1890:117). The animals were allowed to graze only on a small, very dry hillside pasture. Curtice's control of experimental conditions was inadequate, and since we do not know the intermediary host of a single member of this group, his argument that he had excluded the likely hosts falls flat.

Zurn stated that infestation is common in stabled sheep, but Flattely was informed that stabled sheep in the Marches of Ancona, Romagna,

Perugia and Umbria, were free from infestation. The conditions of stabling, the nature of the floors, the frequency of removal of manure and a host of other factors may vary to such an extent that observations of natural occurrence under such conditions are quite valueless unless they are accompanied by detailed and accurate information on these points.

With regard to actual feeding experiments to demonstrate the possibility of direct transmission, the feeding of mature eggs by Stiles (Stiles and Hassall, 1893) gave only negative results, as did a similar experiment by Flattely (1922). In spite of his conviction that the life-history would prove to be a direct one, Curtice was also unable to demonstrate infection by this method. Mönnig (1929) has fed eggs, fresh and matured by keeping in moist sand, and has failed to obtain infestation in this way. The writer in England also failed to infect by feeding stabled lambs upon ripe segments and matured eggs.

In a recently published paper Sinitsin (1931) has put forward the hypothesis of direct air-borne infection, largely as a result of his observations on the resistance to desiccation of the embryo within its envelopes. He concluded that some 45 days of preliminary development in a moist environment are necessary, and that after this stage has been completed a brief period of desiccation is an essential condition in order to bring about rupture of the egg-shell and liberation of the embryo. As soon as the horns of the embryo come into contact with a moist surface such as the respiratory mucous membrane of a mammal the boring apparatus is set in action and the capsule eventually becomes detached. The respiratory mucus is said to dissolve the capsule in less than 30 minutes.

Sinitsin's conclusions with regard to the actual course of development within the egg are contested by Scott and Honess (1931), who state that under the influence of drying, the embryo shrinks and probably does not survive beyond three weeks. They are also unable to accept Sinitsin's view that the horns of the pyriform apparatus can be used to bore into the tissues of the host.

INDIRECT TRANSMISSION

The more orthodox view that an intermediary host is concerned in the life-history of members of this group has stimulated workers to examine large numbers of possible vectors in infested areas and to carry out direct attempts to infect the most likely species by the feeding of Moniezia eggs. It may be accepted that up to the present no intermediate stage that can definitely be linked with any member of the Anoplocephalid group has been discovered in either a vertebrate or invertebrate host. A record of the chief invertebrates examined and

the workers concerned, together with the method employed, is given in Table I.

TABLE I.—*Invertebrates examined as possible intermediate hosts of Moniezia*

EARTHWORMS	*Fridericia pretoriana*	Mönnig 1929	Attempted infestation
SNAILS	*Helix* and *Succinea* spp.	Joyeux 1920	Attempted experimental infestation
SLUGS	Undetermined spp.	Grassi & Rovelli 1892	Examined for infestation
	Agriolimax agrestis	Lebour 1915, Flattely 1922, Daubney 1923	Both methods
	Arion circumscriptus	Lebour 1915	Attempted infestation
	Arion hortensis	Flattely 1922, Daubney 1923	Examined for infestation
SPIDERS	6 spp. unidentified	Mönnig 1929	Attempted infestation
MITES	*Amolops* sp.	"	"
	Glyciphagus sp.	"	"
WOODLICE	Unidentified	"	"
CENTIPEDES	Chilopoda unidentified	"	"
MILLIPEDES	Diplopoda unidentified	"	"
SPRINGTAILS	Collembola unidentified	"	"
GRASSHOPPERS Acrididae	*Ochrophlebia ligniola;* four other spp.	"	"
CRICKETS Gryllidae	*Liogryllus bimaculatus, Gryllus* sp.	"	"
HEMIPTERA	2 spp. unidentified	"	"
Reduviidae	Coranus, 2 spp. unidentified	"	"
LEPIDOPTERA	Caterpillar unidentified	"	"
COLEOPTERA			
Carabidae	*Harpalus cursorius, H. claviceps,* 5 spp. unidentified	"	"
Hydrophilidae	*Cercyon haemorrhoidale, C. melanocephalum*	Daubney 1923	"
Staphylinidae	*Aleochara lanuginosa*	Flattely 1922	Examined for infestation
Histeridae	*Hister carbonarius*	Daubney 1923	Attempted infestation
	Triballus ascaphus	Mönnig 1929	"
Scarabaeidae	*Onthophagus vacca*	Joyeux 1920	"
	Onthophagus fracticornis	Daubney 1923	"
	Aphodius erraticus	"	"
	Aphodius fimetarius	Joyeux 1920, Daubney 1923	"
	Aphodius ater	Flattely 1922	Examined for infestation
	Aphodius granarius	Joyeux 1920, Daubney 1923	Attempted infestation
	Aphodius punctatosulcatus	Flattely 1922	Examined for infestation
	Aphodius prodromus	Flattely 1922, Daubney 1923	Both methods
	Aphodius rufipes	Daubney 1923	Attempted infestation
	Oxyomus porcatus	Daubney 1923	"
	Geotrupes sylvaticus	Joyeux 1920, Joyeux & Kobozieff, 1928	Both methods
Ptinidae	*Trigonogenius globulum*	Mönnig 1929	Attempted infestation
Crioceridae	*Lema bilineata*	"	"
Chrysomelidae	*Ageniosa* sp.	"	"
Tenebrionidae	*Trigonopus typhon*	"	"
	Gonocephalum simplex	"	"
Cydnidae	*Cydnus lepidus*	"	"
ANTS	2 spp. not identified	"	"
DIPTERA			
Cordyluridae (Dung flies)	*Scatophaga stercorarius*	Flattely 1922	Examined for infestation

It has been suggested that an intermediary host might be found among (a) the ectoparasites of sheep (b) among blood-sucking insects

or arthropoda (c) among small animals that may be ingested by sheep during grazing or (d) among larger species whose faeces can soil pastures over which sheep graze.

ANTE- OR POST-NATAL INFESTATION

The early age at which a heavy incidence of infestation can be detected in flocks has given rise to the view, extremely popular with laymen, such as farmers and shepherds, that infestation normally takes place before birth. There is obviously no point at present in debating whether or not ante-natal infestation is a possibility. The point at issue is what constitutes the normal method of infestation, and in this connection it is relevant to remark that while there are no records of newly-born lambs having tapeworms at the time of birth there is ample proof that infestation takes place freely after birth, as shown by the comparative frequency with which extremely young tapeworms (5 mm. or less in length) are collected from sheep over a year old.

The alternative already mentioned, that lambs may acquire infestation from the milk of the ewe, or from the exterior of the udder, has been considered by Flattely (1922) who examined the centrifuged deposit from the milk of ewes from a heavily-infested flock. It is interesting also to note that Flattely found in sections of the udder of a ewe bodies superficially resembling hexacanth embryos, which later he became convinced were stages in the life-history of some protozoan parasite.

In a recent publication Konsuloff (1929) has described and figured somewhat indefinite structures which he considers to be larval stages of Moniezia from the milk of ewes and cows. On the basis of his findings Konsuloff advances the hypothesis that eggs of Moniezia, ingested by an adult ruminant, leave the alimentary tract and pass to the region of the udder, where they undergo some development, and are finally excreted with the milk. He evidently considers the sojourn of the immature parasite in the adult host to be but a brief one and concludes that lambs are only liable to contract infection during the period when their lactating mothers are ingesting eggs from the pastures, soon after being released from their winter stables. According to Konsuloff lambs and calves may be infested in similar fashion by drinking the milk of grazing cows, and he relates that he has found larval stages of the parasite in cows' milk also at this season of the year.

If it were true that Moniezia of sheep are normally transmitted to young lambs in the milk it would be unlikely that this particular method of transmission should be peculiar to the tapeworms of ruminants, and one would expect to find the same method operating in the life-cycles

of other species of the Anoplocephalidae. There is unfortunately very little known of the life-histories of other members of the group, but in his study of the cestodes of the pocket-gopher Douthitt (1915) found that the pocket-gopher was never infested under the age of 8 months, a fact which points rather to a seasonal infestation dependent upon the seasonal occurrence of some intermediate host or upon the annual recurrence of seasonal conditions suitable for the direct development of the parasite. One might also draw attention to the frequency of records of Anoplocephala in aged horses.

MUZZLING EXPERIMENTS

A muzzling experiment carried out on highly infected pastures should yield much valuable information if successful. Such an experiment, designed by Mönnig, failed on account of the unusually dry conditions that obtained at the time of the experiment, only one animal out of 20 unmuzzled controls acquiring infestation.

The present writer's muzzling experiment, to which Mönnig refers in an appendix to his paper, was carried out in 1923. Twelve lambs were obtained from a hillside farm in the North of England, on which tapeworm of sheep was believed not to occur, and were removed from their mothers at birth. They were fed on cows' milk and were transported to the South of England to be placed on a small two and one-half acre pasture, on which heavy infestations had been contracted both during the previous year and earlier during the same season. Since the lambing period on the northern farm was roughly two and one-half months later than that on the southern one the experimental lambs were moved on to the infected pasture at a considerably later date than was normal on this particular farm. Of the 12 lambs obtained one died during the railway journey. Five of the remaining 11 lambs were fitted with leather muzzles before they were released on the pasture. The muzzles resembled an ordinary dog-muzzle having an almost hemispherical cap of solid leather at the tip approximately 3 inches in diameter; the remainder of the muzzle was formed by a lattice-work of leather thongs. The other 6 lambs were released on the pasture without muzzles, and no other animals were allowed to graze over the area. All the lambs were fed on cows' milk only, at first by bottle and later from buckets, the muzzles being removed in a small shed where the feeding was carried out. One of the unmuzzled controls died during the first week of exposure.

Fecal samples were examined weekly for the presence of helminth eggs. All five unmuzzled lambs contracted infestations with Moniezia and showed transient infections with coccidia, and the three that were allowed to survive longer than seven weeks showed in addition light

nematode infestations. The first positive results were obtained at the fecal examination on the 52nd day of exposure when 2 of the controls showed eggs of Moniezia. These 2 were killed during the following week and were found each to contain several specimens of *M. expansa,* some of which measured over one yard in length. One hundred and fifty-seven days from the commencement of the experiment all three of the remaining unmuzzled controls showed eggs of Moniezia together with light nematode infestations, and they were killed and the presence of Moniezia confirmed by collection of the worms. The exact dates on which these animals first showed infestation cannot unfortunately be given since detailed records were left behind in England. Throughout the period of observation the 5 control animals remained in good condition.

The five muzzled animals remained on the pasture for nearly three months longer without ever showing any evidence of helminth infestation. Coccidia were never found in the faeces of these animals. During this period the muzzles were twice exchanged for larger ones. At the close of the experiment the muzzled animals were slaughtered and the contents of the alimentary canal were examined by the washing and sedimentation method. In none of the five could cestodes or nematodes be discovered.

The clean-cut result obtained in this experiment was considered to justify the following conclusions:—

(a) That post-natal infestation of lambs is not only possible, but commonly occurs.

(b) That infestations are contracted by ingestion during grazing.

(c) That the milk of the ewe or cow is not an essential vehicle for the infective stages.

(d) That infestation is not conveyed by the bite of blood-sucking insects or arthropods.

(e) That infection is not air-borne.

(f) That it is unlikely that infestation is contracted by eating ectoparasites, since there was ample opportunity to eat parasites when the muzzles were removed for feeding. The only ectoparasites present were keds, which were present on all the animals.

It is accordingly no longer necessary to seek a solution along unorthodox channels. The hypothesis recently revived by Konsuloff concerning the transference of the infective stages in the milk of the ewe and cow, and that of Sinitsin that infection is air-borne may therefore be discarded.

The following possibilities remain-unaffected:—

(1) The ingestion of the infective stages in small animals or even in plants that may be eaten during grazing.

(2) The ingestion of the infective stages derived from the faeces of animals acting as intermediary hosts.

(3) The direct ingestion of matured eggs remaining on the pastures. This is the least probable explanation.

It was originally planned to continue this experiment along certain lines that were considered to offer the greatest chance of success, but the program was abandoned when the writer left England. It was intended to liberate young uninfested lambs on this small area at intervals throughout the year and in this way to find out whether the infestation could be contracted all the year round or whether there was any seasonal variation. The next step countemplated was the subdivision of the already small area to ascertain in how small a division infestation could be contracted.

The smallest infective plots could almost literally be removed to the laboratory and catalogued as to flora and fauna likely to be of importance in the life-history of Moniezia. It is still the writer's opinion that once it has been definitely established that heavy infestations can regularly be contracted on one small pasture the best method of approach to the problem will be along these lines. The method will be more likely to yield successful results in a country that experiences marked seasonal variations of climate, which may affect the distribution or activity of the intermediary host.

References Cited

Curtice, C. 1890.—The Animal Parasites of Sheep. Washington, D. C. 222 pp., 36 pl.

Daubney, R. 1923.—The Adult Tapeworms of Sheep, particularly those occurring in Great Britain. (With discussion.) Vet. Record, 3:679–686.

Douthitt, H. 1915.—Studies on the Cestode Family Anaplocephalidae. Ill. Biol. Monogr., 1:355–446, 6 pl.

Flattely, F. W. 1922. Considerations on the Life-History of Tapeworms of the Genus Moniezia. Parasit., 14:268–281.

Grassi, G. B., and Rovelli, G. 1892.—Ricerche embriologiche sui Cestodi. Att. Accad. Gioenia Sci. Nat. Catania, Ser. 4, (Mem. 2), 4:1–108, 4 pl.

Joyeux, C. 1920.—Cycle evolutif de quelques Cestodes. Bull. Biol. France, Belg., Suppl. 2:1–216, 7 pl.

Joyeux, C., and Kobozieff, N. I. 1929.—Recherches sur l'evolution des cestodes de la famille des Anoplocephalidae. Ann. Parasit., 7:477–482.

Konsuloff, S. 1929.—Recherches sur l'evolution des cestodes de la famille des Anoplocephalidae. Ann. Parasit. Hum. Comp., 7:477–482.

Lebour, M. 1915.—Some Feeding Habits of Slugs. Ann. Applied Biol., 1:393–395.

Mönnig, H. O. 1929.—Investigations into the Life-History of the Tapeworm *Moniezia expansa*. Ann. Rep. Dir. Vet. Serv. Union So. Africa, 15:317–327.

Scott, J. W., and Honess, R. F. 1931.—A Study of the Eggs of *Moniezia expansa*. Jour. Parasit., 18:130 (abstract).

Sinitsin, D. F. 1931.—A Glimpse into the Life History of the Tapeworm of Sheep, *Moniezia expansa*. Jour. Parasit., 17:223–227, pl. 21.

Stiles, C. W., and Hassall, A. 1893.—A Revision of the Adult Cestodes of Cattle, Sheep, and Allied Animals. Bull. Bur. An. Ind., 4, 103 pp., 16 pl.

THE TRANSITION OF *TROGLODYTELLA ABRASSARTI* AND *TROGLODYTELLA ABRASSARTI ACUMINATA*, INTESTINAL CILIATES OF THE CHIMPANZEE, FROM ONE TYPE TO THE OTHER

W. W. Swezey*

During the winter of 1930–31 a species of Troglodytella, tentatively identified as *Troglodytella abrassarti acuminata* Reichenow, 1917, by Nelson (1932) was found to be harbored by some chimpanzees kept at the Johns Hopkins University. This was the sixth report of these ciliates in the literature, the first being the original description by Brumpt and Joyeux (1912) who gave the organism the genus and species name *Troglodytella abrassarti.* The second and third reports were by Reichenow (1917 and 1920). He added another species, *Troglodytella gorillae,* to this genus, which he found in a gorilla and considered to differ sufficiently from the original to be given a new specific name. The form that he discovered in the chimpanzee had a longer and more pointed posterior end than that described and illustrated by Brumpt and Joyeux. He decided that it was sufficiently different to permit the creation of a subspecies, *Troglodytella abrassarti acuminata.* This genus was next mentioned by Kirby (1928) who noted it in four chimpanzees kept at Yale University. The fifth account of this genus in the literature was by Curasson (1929) who claimed a pathogenic rôle for it, and the sixth by Nelson (1932) who tried to cultivate it on artificial media.

Reichenow stated that the only difference between *Troglodytella abrassarti* and *Troglodytella abrassarti acuminata* was the fact that the latter had a much longer and more pointed posterior end than the former. Buisson (1923) suggested the possibility that the infusorium described by Brumpt and Joyeux might have been contracted, which would explain why they did not see the long, pointed posterior end which Reichenow describes and illustrates.

During the course of routine examination, more than 350 having been made, of the chimpanzees kept at the Johns Hopkins University, 11 in number, 7 of which harbor the parasite, it was noticed on several occasions that practically all of the living and actively moving forms in the smears, were of the form described by Brumpt and Joyeux as *Troglodytella abrassarti.* They had a blunt, rounded posterior end or

* From the Department of Protozoology of the Johns Hopkins University, School of Hygiene and Public Health.—This work was aided by a grant from the Committee on Scientific Research of the American Medical Association.

"tail," when one was present at all. After a period of a few hours the stools were examined again and practically all of the forms were now more or less quiescent and possessed a long pointed posterior end corresponding to that of the subspecies described by Reichenow as *Troglodytella abrassarti acuminata*. These observations led to experiments to determine whether a change or transition from one form to another takes place.

The stools were obtained as fresh as possible and placed in the incubator at 37.5°C, after tap water had been added to them. Enough tap water was added to keep the stool moist, but without entirely immersing it. It was found by several experiments that in tap water, which serves to keep the stool moist at higher temperatures, the ciliates will remain alive and active for a longer period of time than when Ringer's solution or normal saline (0.85%) is added. The temperature was also found to be optimum at 37.5°C. The specimens of Troglodytella in stools examined at half hour intervals for three hours showed a gradual transition from an almost completely acaudate form to a very pronounced caudate form in which the tail was long and pointed. All of the specimens present became modified in the same way.

In all cases, apparently, the more active the organism the less its probability of having a tail, for the more active of the ciliates were either acaudate or had a blunt, rounded posterior end. On some occasions it was noted that after the organisms had become quiescent and more or less caudate, the heating of the slide to stimulate their action would also cause a shortening of the posterior end, even to the point of disappearance in some cases. The addition of warm water or warm normal saline to the smear also stimulated the beating of the cilia so that the posterior end would contract. This observation lead to the hypothesis that the posterior end is a contractile organelle which can be drawn inside the body or protruded as a result of some stimulus, possibly the movement of the cilia, which in turn may be stimulated by the medium in which the organism is situated, or by heat, or both. Sections of the infusorium are being prepared in order to determine whether or not this "tail" is a contractile organelle.

Single individuals were followed while this transition was taking place and it required approximately 30 minutes to complete the change. Camera lucida drawings were made of the posterior end at intervals of 5 minutes and are shown in Figs. 3 to 8. Brumpt and Joyeux's illustration of *Troglodytella abrassarti* and Reichenow's drawing of *Troglodytella abrassarti acuminata* are included to show their similarity to different stages in this transition process. Note the similarity of the

posterior end of Brumpt and Joyeux's form (Fig. 1) to Fig. 4, and of Reichenow's variety (Fig. 2) to Fig. 7.

The significance of these observations can be seen when one realizes that two species of Troglodytella and the supposed variety of one of these are three of the four Protozoa that have been described from apes and not from man (Hegner, 1928). The other protozoon is *Babesia pitheci.* So, instead of two species of Troglodytella and a variety, these observations show that, in reality, there are only the two species of Troglodytella and *Babesia pitheci,* making a total of *three* Protozoa that have been described from apes and not from man.

Fig. 1

Fig. 2

Text Figure 1.—*Troglodytella abrassarti,* after Brumpt and Joyeux. Notice the similarity of the posterior end to Fig. 5.

Text Figure 2.—*Troglodytella abrassarti acuminata,* after Reichenow. Notice the similarity of the posterior end to Fig. 8.

Further experiments may also show that the species *Troglodytella gorillae* is not a good species, and may fall under the species *Troglodytella abrassarti.*

Thus, the change from the form of Brumpt and Joyeux to the subspecies described by Reichenow has been observed, which shows that *Troglodytella abrassarti* and *Troglodytella abrassarti acuminata* are both stages in a transition from an acaudate to a caudate form. Therefore the foundations for the formation of this variety, or subspecies, are groundless, since they both occur as forms assumed by a single individual.

Up to the present time it has been possible for the writer to examine only two gorillas and neither of these was infected with Troglodytella. *Troglodytella gorillae* may be rare since no one seems to have observed it excepting its discoverer, Reichenow (1917).

These observations may have some bearing upon the specificity of

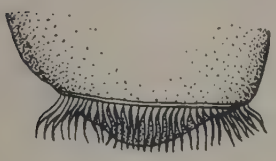


Fig. 3

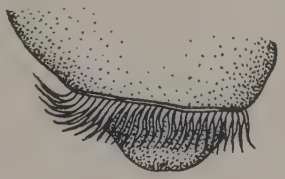


Fig. 4

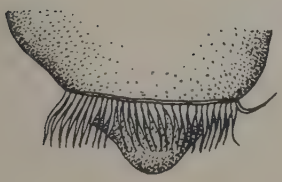


Fig. 5

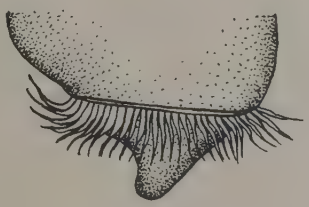


Fig. 6

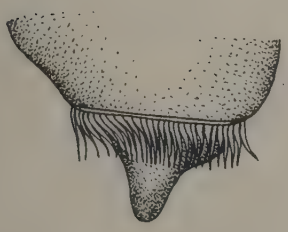


Fig. 7

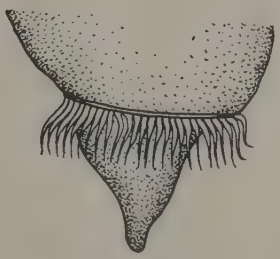


Fig. 8

Text Figures 3–8.—Transition stages of *Troglodytella abrassarti* from an acaudate to a caudate form. Camera lucida drawings made at 5 minute intervals.

certain ciliates that have been described from ruminants and which differ from certain others only in the form of the posterior end. As a result of the proper stimulus they might change to another form with a posterior end different in shape and structure.

ACKNOWLEDGEMENTS

The writer wishes to express his indebtedness to Drs. Van Volkenburgh and Long and the Committee on the Cold Research, from whose chimpanzees the material for this work was obtained; also to Drs. Hegner and Andrews for their helpful suggestions and criticisms.

BIBLIOGRAPHY

Brumpt, E., and Joyeux, Ch. 1912.—Sur un Infusoire nouveau parasite du Chimpanzé Troglodytella abrassarti, n.g.n.sp. Bull. Soc. Path. Exot. 5: 499–503.

Buisson, Jean. 1923.—Les Infusoires Ciliés du Tube Digestif de L'homme et des Mammifères.—Trav. Lab. Parasitol. Fac. Med. Paris, 132–138.

Curasson, G. 1929.—Troglodytella abrassarti Infusoire Pathogene du Chimpanzé. Ann. de Parasitol. 7:466–468.

Hegner, Robert. 1928.—The Evolutionary Significance of the Protozoan Parasites of Monkeys and Man. Quart. Rev. Biol. 3:225–244.

Kirby, Harold, Jr. 1928.—Notes on Some Parasites from Chimpanzees. Soc. Exp. Biol. and Med. Proc. 25:698–700.

Nelson, E. C. 1932.—The Cultivation of a Species of Troglodytella, a large Ciliate from the Chimpanzee. Science, 75:317–318.

Reichenow, E. 1917.—Parasitos de la Sangre y del Intestino de los Monos Antropomorfos Africanos. Bol. R. Soc. Esp. de Historia Natural. 17:312–332.

Reichenow, E. 1920.—Den Wiederkäuer—Infusorien verwandte Formen aus Gorilla und Schimpanse. Arch. f. Protistenk. 41:1–33.

ASCARIS LARVAE AS A CAUSE OF LIVER AND LUNG LESIONS IN SWINE

BENJAMIN SCHWARTZ and JOSEPH E. ALICATA
Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C.

In a previous paper the writers (1930) reported the occurrence of lesions in the lungs and liver of swine in the form of small pearly cysts under the pleura of the lungs and under the capsule of the liver. While practically each cyst isolated from the lungs contained a brittle, degenerated nematode larva, no parasites were discovered in several liver cysts which were subjected to a searching microscopic examination. More recently, the writers had an opportunity to examine a liver of a pig which had been killed at the Bureau of Animal Industry Experiment Station at Bethesda, Maryland. The liver showed lesions essentially similar to those noted on the previous occasion except that the lesions were more numerous and therefore more striking. From a number of the discrete pearly cysts, located underneath the capsule, degenerated nematode larvae, morphologically identical with those reported previously from the lungs of swine, were isolated for study. This finding completed the chain of evidence, already suspected from the earlier study, that small cysts in the liver and lungs of swine frequently encountered by veterinary inspectors and others in the course of post-mortem examination of these animals, are due to the encystment and ultimate degeneration of nematode larvae. While the lesions in the lungs are limited to the cysts themselves, those in the liver are more striking because the entire organ takes on a mottled appearance, due to the striking contrast between the normal color of this organ and the pale color of the cysts; the contrast is particularly marked when the cysts are numerous.

MACROSCOPIC APPEARANCE OF LESIONS

On macroscopic examination the liver presents a striking mottling, the mottled areas being grayish or whitish in appearance. In certain regions of the liver, more particularly along the borders, as may be noted in figure 1, discrete circular spots, whitish in appearance, are readily noted. In other portions of the liver, the whitish areas are more diffuse, due perhaps to the confluence of two or more adjoining whitish spots, and to the extension of the lesions beyond their more normal limits, owing to the extension of the irritation.

When dissected from the liver tissue, the discrete whitish lesions are seen to be due to more or less spherically shaped cysts located

immediately underneath the capsule, each cyst containing a granular material. The more diffuse lesions are due in part to an increase in the interlobular connective tissue. As already noted, some of the cysts, particularly those along the margin of the liver, contain nematode larvae, only one larva being contained in a single cyst. Figure 2 illustrates the appearance of a cyst, when flattened under cover glass pressure and viewed as a wet preparation under the microscope. It will

Text Figure 1.—Photograph of liver, showing lesions due to Ascaris larvae. Note discrete whitish areas (cysts) along margins.

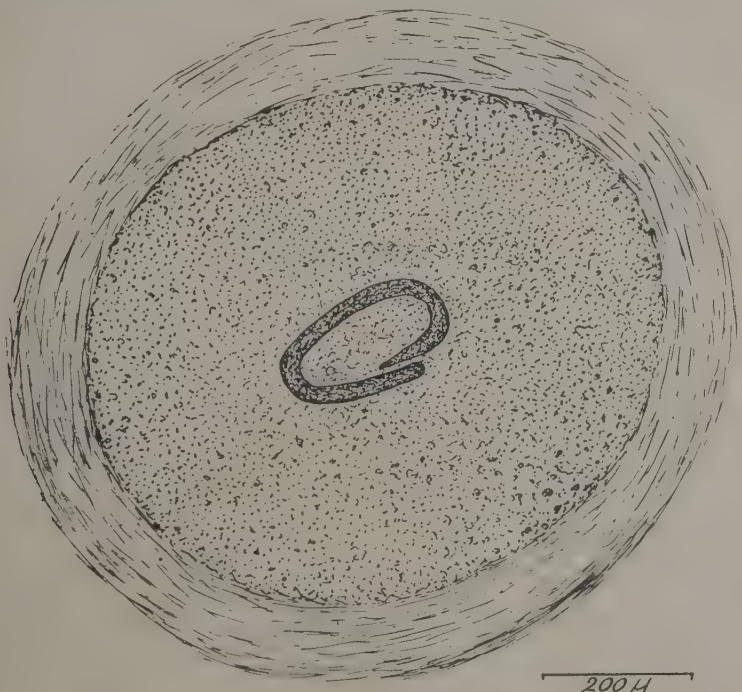
be noted that the cyst wall is rather thick and fibrous; it is in fact composed of connective tissue. The cavity of the cyst contains a granular material, and the larva is more or less centrally located within the granular mass.

The intact cysts observed in the liver and lungs, and already reported by the writers briefly in a previous paper (1930), were from one to two millimeters in diameter. Several of the discrete whitish areas seen through the capsule of the liver, shown in figure 1, measured approximately one millimeter in diameter. When freed from the hepatic tissue, three pearly cysts showed the following measurements: 980 μ

by 820 μ; 760 μ by 640 μ; and 840 μ by 640 μ. Thus it is seen that the cysts proper are not spherical but rather elliptical in shape.

MORPHOLOGY OF LARVAE ISOLATED FROM CYSTS

The larvae isolated from the cysts in the liver were from about 450 μ to 550 μ long and from 15 μ to 18 μ in maximum width. No internal structure could be made out in these worms, the larvae having



Text Figure 2.—Microscopic appearance of isolated cyst. Note thick cyst wall, caseous content of cyst and degenerated Ascaris larva located centrally within cyst.

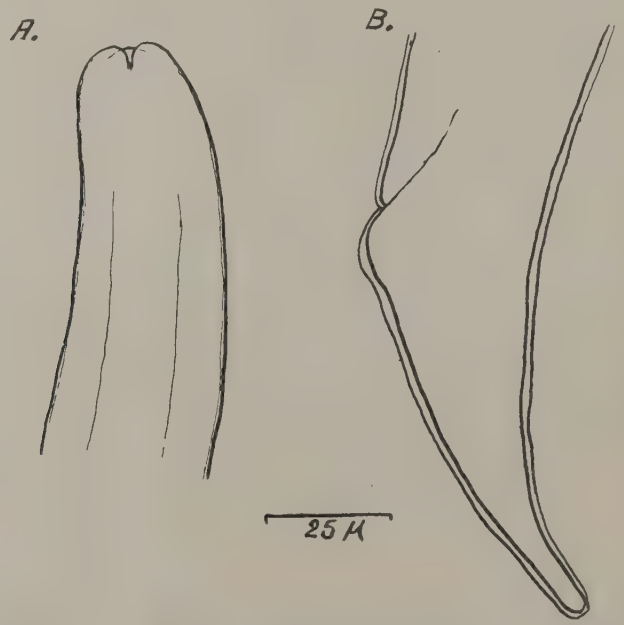
a uniformly granular appearance. It will be noted that the larva shown in figure 2 has a bluntly rounded anterior extremity, a more or less uniform diameter, except in the tail region which tapers gradually, its terminal portion being blunt.

The larvae isolated from the lungs on a previous occasion were larger than those reported for the first time in this paper as occurring in the liver. One larva from the lungs was 1.7 mm. long by 62 μ in maximum width, or about three times the size of the larvae from the

liver. The anterior end of the former, shown in figure 3A, shows three lips, and the posterior end, 3B, shows a tail 92 μ long, directed dorsad and gradually attentuated, with a blunt tip.

SPECIFIC IDENTITY OF THE LARVAE FROM THE LIVER AND LUNGS

While the larvae which have been isolated from cysts in the liver and lungs of swine are morphologically degenerated, it is nevertheless possible to assign them with a fair degree of certainty to the species *Ascaris suum*. The fact that the larvae obtained from the liver are only about half the size of the larvae obtained from the lungs, is significant



Text Figure 3.—Ascaris larva obtained from cyst in lung. A, anterior end; B, posterior end. Note tail of larva directed dorsad.

in this connection, and shows that the parasite in question is one which migrates from the liver to the lungs and increases in size during the period involved in the transfer from the former to the latter organ. As is well known, Ascaris larvae actually undergo such migration in the bodies of swine. Ransom and Foster (1920) state that Ascaris larvae grow in the lungs and attain a length 5 to 10 times that of the newly hatched larvae. These writers give an illustration of larva 1.43

mm. long, with a tail 80 μ long. The ratio of the length of the tail to the total body length in this larva is approximately the same as that in the degenerated larva referred to above as having been obtained from the lungs of a pig. What is perhaps a more striking resemblance than mere identity in a certain size relationship, is the morphology of the tail of the larva shown in figure 3B. The tail is directed dorsad, a feature also shown in the illustration of an Ascaris larva obtained by Ransom and Foster (1920) from the lungs of an experimental animal. These writers also noted that Ascaris larvae die and degenerate in the course of their migrations. In this connection, they make the following statements:

"That larvae die in the liver is evident from finding dead and degenerated larvae in the liver of a mouse which was killed 296 days after it had been fed *Ascaris suum* eggs. There was a small encapsulated tumor near the margin of the liver, containing a caseous material and several dead and degenerated Ascaris larvae. On several occasions dead and degenerated larvae have been found in the lungs, for example in a rabbit 86 days after infection and in a pig 65 days after infection. It is quite probable that the larvae that migrate to the spleen, thyroid and other organs outside the usual path of migration ultimately die and become encysted and absorbed or perhaps in some cases stimulate the surrounding tissues to other reactions."

While the possibility that the larvae discovered by the writers in liver and lung lesions of pigs might belong to a species other than *Ascaris suum* cannot be absolutely excluded, the available evidence certainly does not indicate that the larvae in question are other than Ascaris larvae. The only nematode larvae other than those of Ascaris which invade the livers of swine with regularity are those of the kidney worm, *Stephanurus dentatus*. Aside from the fact that the latter are morphologically distinct from Ascaris larvae, they are known to produce large areas of fibrosis which extend deeply into the hepatic tissue. The liver lesions found in association with the larvae discussed in this paper are superficial and are characterized by a general mottling of the liver surface and not by large areas of fibrosis. Moreover, *Stephanurus* larvae do not, so far as is known, encyst and then degenerate in the early stages of their development. Larvae of *Stephanurus* found in states of degeneration have usually progressed much farther in development than those shown in the illustrations given in this paper. The liver is a normal temporary habitat of *Stephanurus;* these worms undergo considerable growth and development in that organ and remain in it for weeks, only those larvae degenerating which fail, for one reason or another, to extricate themselves from the hepatic tissue by

perforating the capsule. These facts were determined by Schwartz and Price (1929).

Another possibility which cannot be overlooked altogether is that the larvae in question might be those of *Oesophagostomum*. The latter, to be sure, have not been reported from the liver of swine. As larvae, they occur encysted in the wall of the large intestine. However, the fact that these larvae penetrate the intestinal wall, would naturally lead to the possibility of their reaching the liver, at least occasionally. However, aside from the fact that *Oesophagostomum* larvae have never been reported from the liver of swine, their morphology at stages corresponding to those reported by the writers from the livers and lungs of pigs, warrants the conclusion that the larvae discussed and figured in this paper are not those of *Oesophagostomum*.

DISCUSSION OF LIVER LESIONS IN SWINE DUE TO NEMATODES

Swine livers are commonly observed to show various pathological conditions which necessitate either trimming or condemnation under meat inspection procedure. Schwartz and Price (1929) showed rather conclusively that certain lesions of common occurrence in the livers of swine observed at various abattoirs operating under government supervision can be experimentally produced in kidney worm free pigs by infecting the latter with the larvae of these parasites. In addition to kidney worm lesions in the liver, lesions similar to those described in this paper for the first time as being due to Ascaris larvae, have been brought to the attention of the Zoological Division from time to time by various bureau inspectors in charge of meat inspection and by others. In the past, attempts to find the cause of this condition have proven fruitless, although Ascaris larvae have been suspected of being the etiological agents of this condition. Hall (1924) makes the following statement regarding Ascaris larvae as a probable cause of liver lesions in swine: "They cause lesions in the liver and the common parasitic cirrhosis in swine livers may yet be shown to be due, at least in part, to these larvae." Microscopic sections of the lesions in question by the writers and by other workers in the Federal Bureau of Animal Industry have always revealed an increase in the interlobular connective tissue, usually accompanied by an accumulation of eosinophile leucocytes in the region of the lesions. Aside from these findings, nothing else of significance has been noted. The present report embodies, therefore, the first concrete evidence that lesions of the sort described in this paper and at least some lesions referred to by Hall as "the common parasitic cirrhosis of swine livers" are associated with, and are, doubtless, caused by larvae of Ascaris which have become arrested in the

liver and have subsequently become encapsulated and ultimately degenerated. The fact that various livers examined by the writers on numerous occasions showed lesions essentially similar to those noted in this paper, including the presence of discrete cysts free from worms, is evidence that the larvae in the cysts ultimately disintegrate beyond recognition.

The finding of a liver in which the larvae had not completely degenerated was a happy circumstance which enabled the writers to clear up a cause of lesions of common occurrence in the livers of swine in the United States. In this connection it should be noted that swine livers showing a superficial mottling or fibrosis have been received at various times in the Bureau of Animal Industry from various localities in the United States where kidney worm infestation is not known to exist, but where Ascaris is certainly present, if not widely prevalent. The epidemiological evidence of this liver disease thus fits in nicely with the known distribution of Ascaris in swine, but not with the known distribution of the swine kidney worm.

SUMMARY

Lesions observed from time to time in the liver and lungs of swine were found to be due to the presence of small pearly cysts, from one to two millimeters in diameter, when measured while in *situ*.

Each cyst contains a granular material and also shows at times, though not invariably, a nematode larva in a state of evident degeneration.

The larva is brittle and has a uniformly granular appearance. It is indistinguishable morphologically from the corresponding stages of *Ascaris suum* as described by Ransom and Foster.

The lung lesions are limited to the presence of the pearly cysts, while the liver lesions are more striking and appear as a marked mottling of the surface of this organ. The mottling is due in part to an increase of interlobular connective tissue, and in part to the presence of cysts underneath the capsule. Liver lesions indistinguishable from those which contain discrete cysts and larvae in the cysts, are of common occurrence in swine in this country.

The larvae evidently disintegrate in the cysts, apparently earlier in those in the liver than in those in the lungs, which accounts, in all probability, for the presence in swine livers of discrete cysts not containing worms.

The geographical distribution of liver lesions in swine, similar to those described in this paper as being due to Ascaris larvae, makes it probable that these worms are responsible for this condition.

References to Literature Cited

Hall, Maurice C. 1924.—Worm Parasites of Domesticated Animals. Parasites of Swine. 1–160.

Ransom, B. H., and Foster, W. D. 1920.—Observations on the life history of *Ascaris lumbricoides*. U. S. Dept. Agric. Bull. 817, 1–47.

Schwartz, Benjamin and Alicata, J. E. 1930.—On the occurrence of encysted nematode larvae in the liver and lungs of swine. Jour. of Parasitology, 16:168.

Schwartz, Benjamin, and Price, E. W. 1929.—The life history of the swine kidney worm. Science, 70:613–614.

A NEW SPECIES OF LONGISTRIATA (NEMATODA) FROM THE COTTON RAT, *SIGMODON HISPIDUS,* WITH NOTES ON THE DIVISION OF THE HELIGMOSOMINAE INTO GENERA

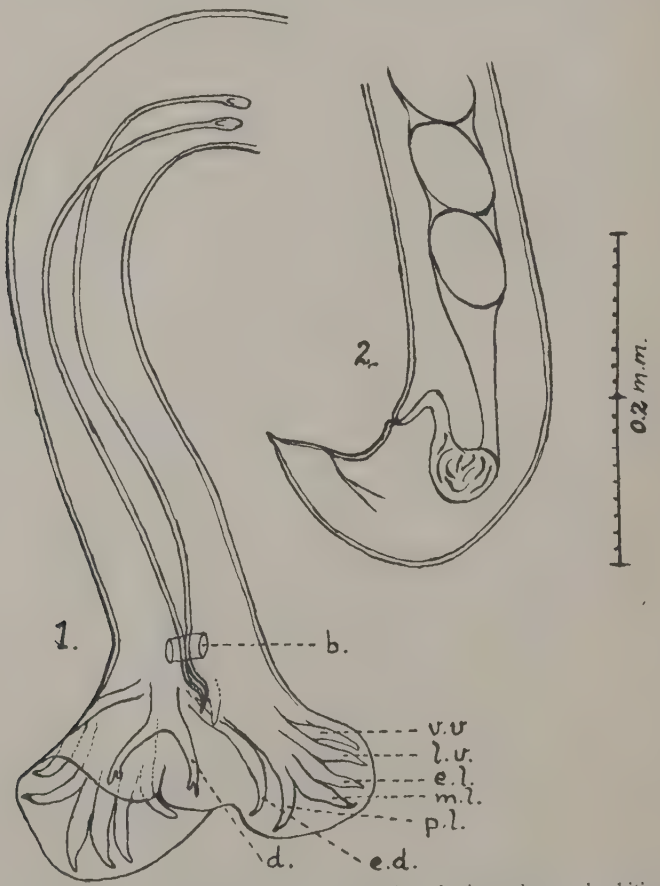
Asa C. Chandler
Rice Institute, Houston, Texas

Cotton rats, *Sigmodon hispidus,* from the vicinity of Houston, Texas, are almost invariably infested with a small intestinal nematode belonging to the genus Longistriata, and constituting a new species for which the name *Longistriata adunca* n. sp. is proposed.

The worms are blood red in color and inhabit the upper one-third of the small intestines of the host. The females reach a length of 4 to 4.9 mm. while the males measure from 3.2 to 4 mm. in length. The maximum width of the females is about 100 to 110 μ, and of the males 65 to 70 μ. The cuticle has fine transverse striations and inconspicuous longitudinal ridges, as well as lateral alae. The cuticle of the head is expanded, and the head is marked off by a constriction in the neck region about 75 μ from the anterior end in the males, and 75 to 84 μ in the females. The outside diameter of the head, i. e., including the cuticle, is 30 μ in the males and 32 to 35 μ in the females, while the inside diameter, exclusive of the cuticle, is 20 μ in the males and 21 to 22 μ in the females. There is an extremely short buccal capsule, surrounded by a ring of minute papillae. The oesophagus has a total length of about 290 to 300 μ, and has a division mark, behind which it is slightly expanded, about 120 to 130 μ from the anterior end.

The bursa of the male (Fig. 1) is symmetrical, and consists of two large lateral lobes and a smaller dorsal lobe which is split in the mid-dorsal line between the two forks of the dorsal ray. The dorsal ray has a very thick trunk at the base and splits in the shape of a letter U into two forks which are bifid at the tips. The externo-dorsal rays come off from near the base of the dorsal ray, though their actual junction with it could not be discerned; they are long and about equal in thickness to the lateral rays, and terminate in the lateral lobes just lateral to the notch which demarcates the dorsal from the lateral lobes. The postero-lateral ray is divergent from the other lateral rays, and bends towards the dorsal lobe. The medio-lateral and externo-lateral rays, together with the latero-ventral, form a group of nearly parallel rays, of approximately equal thickness, and close together. The ventro-ventral is distinctly separated and divergent from the latero-ventral. No prebursal rays were observed. The anus is situated on a prominent

genital cone. The spicules are long and slender, equal in length, and measure 425 to 450 μ in length by about 4 μ in width. They are expanded at the open proximal end, and terminate in a bent point. Near their distal end, just anterior to the bursa, they pass through a con-



Text Figure 1.—Posterior end of male of *Longistriata adunca*. b, chitinous ring; d, dorsal ray; ed, externo-dorsal ray; el, externo-lateral ray; lv, latero-ventral ray; ml, medio-lateral ray; pl, postero-lateral ray; vv, ventro-ventral ray.

Text Figure 2.—Posterior end of female of *Longistriata adunca*.

spicuous chitinous ring. An accessory piece is present, and measures about 30 μ in length.

The female has the posterior end of the body bent ventrally in the shape of a hook; it ends conically, and is terminated by a minute spine 5 to 7 μ long. The anus is about 30 μ from the posterior end, and the vulva about 84 to 88 μ, measured along the ventral contour. The oviduct turns forward from the vulva, and then backward to enlarge into a chamber which is thrown into conspicuous folds, and is evidently part of an ovejecting device. The single uterus runs forward from this chamber, and contains, in its posterior part, a row of about a dozen developed eggs, placed in single file. The eggs measure 72 μ to 76 μ in length by 38 to 39 μ in breadth, and are in a segmented condition when passed from the body of the host.

Location and Host: upper part of small intestine of *Sigmodon hispidus* (cotton rat).

Type locality: Houston, Texas.

Types and cotypes deposited in the helminthological collections of the U. S. National Museum.

There has been considerable confusion relative to the division of the subfamily Heligmosominae into genera, and a considerable number of genera, some of which are almost certainly not valid, have been proposed. The genera have been based largely on bursal characteristics and the development and arrangement of the bursal rays. The presence or absence, or degree of development, of transverse and longitudinal striations of the cuticle, and the degree of development of lateral alae have been given prominence by some writers. The permanent coiling of the body in a spiral has also been emphasized as a character, but all of the species tend to roll into spirals after the host has died and become cold, and it is questionable whether any of them are so permanently coiled as to be unable to straighten out. At any rate it is a character which probably cannot be relied upon.

In surveying the numerous species of the group it seems apparent that some of the characters of the bursa and bursal rays are of importance while others are too variable in obviously closely related species to be given much value. The number and position of the bifurcations and digitations of the dorsal ray, for instance, seem to be too variable to be used as basic generic characters. However, the members of the subfamily seem to fall into two groups with respect to the degree of development of the dorsal ray as a whole. In one group, including the genera Heligmosomum (formerly Heligmosomoides), Nippostrongylus, Nematospira, and Nematospiroides, the dorsal ray is very small and inconspicuous, not nearly reaching the margin of the bursa unless the latter is very deeply incised dorsally. In the other group, of which Longistriata and Viannaia are the outstanding genera, the dorsal ray is large and conspicuous, reaching to, or nearly to, the bursal margin.

Asymmetry of the bursa, when it involves the arrangement of the rays as well as the size of the lobes, is probably worthy of consideration as a generic character. Nippostrongylus, Lane 1923, Nematospiroides, Baylis 1926 (synonym, Sincosta, Roe, 1929), Heligmonina, Baylis 1928, and Heligmonoides, Baylis 1928, are genera in which one of the main features is a marked asymmetry of the bursa, but all of these except Heligmonoides have other characters as well.

The form of the spicules seems to be a good generic character. Viannaia is distinguished by short twisted spicules, while Pudica and Trifurcata, a subgenus of Viannaia, have specially modified spicules with complex terminations; the other genera have long slender spicules ending in simple points.

In addition to these characters there are a number of special characters belonging to certain genera, such as the studding of the cephalic cuticle with small papillae in Impalaia, the enormous development of one lateral ala in Heligmonina, a vulval flap in Heligmostrongylus (here considered a subgenus of Longistriata), a prepuce-like development of the cuticle at the posterior end of the female in Nippostrongylus, a complete doubling of the small dorsal ray in Nematospira, and an abnormal position of the dorsal ray in Nematospiroides.

The type genus of the subfamily is Heligmosomum, Railliet and Henry 1909, with *H. costellatum,* (Duj., 1845) as type species. Later a genus Heligmosomoides, with *H. linstowi,* Hall, 1916, subsequently shown by Boulenger (1922) to be synonymous with *H. polygyrus,* as type was erected by Hall (1916). Travassos and Darriba, 1929, found that *costellatum* was so similar to *polygyrus* as to leave no doubt of their being cogeneric. Heligmosomoides therefore becomes a synonym of Heligmosomum, and most of the species previously referred to Heligmosomum have to be transferred elsewhere.

The genus Viannaia was erected by Travassos, 1914, to include species with a very swollen cuticle, no longitudinal cuticular ridges, short spicules, a forked dorsal ray the branches of which terminate near the margin of the bursa and usually end in six points, and an externo-dorsal ray arising from the trunk of the dorsal. The genus Viannella, Travassos 1918, differs from Viannaia only in minor details of the bursal rays, and should be, in my opinion, considered a synonym of Viannaia. Schulz (1926) divided the genus Viannaia into three subgenera: Viannaia, with transversely striated cuticle without longitudinal striations, and short spicules; Trifurcata, with a cuticle devoid of either transverse or longitudinal striations, and with massive spicules ending in three prongs; and Longistriata, with a transversely striated cuticle with longitudinal ridges, and long slender spicules. The latter subgenus was elevated by Travassos and Darriba (1929) to

generic rank, and most of the species formerly included in the genus Heligmosomum were transferred to it. There is one species, *Viannaia bursobscura,* Dikmans, 1931, which more or less combines characters of three different genera. It has the ventral rays larger than the laterals and long slender spicules, as in Heligmosomum; it lacks longitudinal ridges on the cuticle as in Viannaia; and it has a moderately developed dorsal ray which reaches to near the margin of the bursa, as in Longistriata. However, the bursa is deeply indented dorsally, so that the dorsal rays, which end in four prongs, are rather small and inconspicuous in spite of the fact that they reach nearly to the margin of the bursa. In the writer's opinion the large size of the ventral rays, the small four-pronged dorsal ray, and the long slender spicules, place this species in the genus Heligmosomum in spite of the absence of longitudinal ridges on the cuticle usually present in that genus, though sometimes slightly developed. The form of spicules appears to be a more important character than the longitudinal striations, and this as well as the relative development of the ventral and lateral rays, definitely throws this species out of Viannaia and affiliates it with Heligmosomum. This species is therefore transferred to the genus Heligmosomum.

Heligmosomum agoutii is transferred by Travassos and Darriba to a new genus Füllebornema, on the basis of an unusual form of the externo-dorsal ray and a long dorsal ray. In my opinion the erection of a new genus for this form is not justifiable. The presence of a ventral process or flap over the vulva suggests a relationship to Heligmostrongylus, which also has a large dorsal ray with a lateral prong near the middle of its length, but in this case split clear to its base. I believe that Heligmostrongylus should be reduced to a subgenus of Longistriata, and Füllebornema considered a synonym of it. The species described by Travassos (1918) as *Viannaia pudica* (but figured as "*Heligmosomum pudicus*") is placed by Travassos and Darriba in a new genus Pudica on the basis of the large dorsal ray and the complexity and bifurcation of the tips of the spicules, which are intermediate in length between those of Longistriata and Viannaia.

Walton (1923) erected the genus Nematospira for a species which has short dorsal rays which are completely doubled; otherwise this genus is very close to Heligmosomum. Monnig (1924) erected a genus Impalaia for a form with an aberrant type of dorsal ray, and with the cuticle of the head studded with small papillae. The same author (1927) erected another genus Heligmonella, to which Baylis (1928) added a number of new species. As the genus is recharacterized by Baylis there seems to be no justification for its separation from Longistriata.

The following key should be helpful in placing species of this group in their correct genus. Possibly some of the genera recognized here will have to be suppressed later.

1. Dorsal ray or rays very small, inconspicuous, not nearly reaching margin of bursa 2
   Dorsal ray or rays large, well-developed, reaching to or near to margin of bursa 5
2. Dorsal ray with single root, usually ending in four prongs 3
   Dorsal ray completely doubled ..... Nematospira
3. Bursa symmetrical or nearly so; ventral rays larger than laterals. Heligmosomum
   Bursa asymmetrical 4
4. Ventral rays in both lobes larger than laterals; right and left lobes different in size but similar in arrangement of rays; externo-dorsals much longer than dorsal, with knobs at base ..... Nematospiroides
   Ventral rays not conspicuously larger, if as large, as any of the laterals; right and left lobes of bursa different both in size and arrangement of rays; externo-dorsals little if any longer than dorsals; female with a prepuce-like extension of the cuticle at the posterior end..Nippostrongylus
5. Spicules long, slender, filiform 6
   Spicules short and twisted, or massive, or split into two or three points at tip 9
6. Lateral lobes of bursa symmetrical or approximately so, at least in arrangement of rays 7
   Lateral lobes of bursa markedly asymmetrical both in size and arrangement of rays 8
7. Dorsal ray split to near middle or above middle of its length; cephalic cuticle not studded with papillae ..... Longistriata
   Dorsal ray with a long, thick trunk, thicker than any other ray and bifurcated near tip; cephalic cuticle studded with small papillae ..... Impalaia
8. Left lateral ala enormously developed, as wide as, or wider than, the body proper; externo-dorsals arising near base of dorsals ..... Heligmonina
   Left lateral ala not so highly developed; externo-dorsals arising from trunk of dorsal near the level of its bifurcation ..... Heligmonoides
9. Spicules short, thick and twisted, ending in simple points; ventral rays smaller than laterals ..... Viannaia
   Spicules not as above, bifid or trifid at tip 10
10. Spicules long and massive, trifid at tip ..... Subgenus Trifurcata
    Spicules moderately long, bifid and complex at tip ..... Pudica

The following genera are thrown into synonymy or reduced to subgenera:

Heligmosomoides	synonym of	Heligmosomum
Heligmonella	" "	Longistriata
Füllebornema	" "	Heligmostrongylus
Vianella	" "	Viannaia
Sincosta	" "	Nematospiroides
Heligmostrongylus	subgenus of	Longistriata

Literature Cited

Baylis, H. A. 1926.—On a Trichostrongylid nematode from the woodmouse, *Apodemys sylvaticus*. Ann. and Mag. Nat. Hist., Ser. 9, 18:455–464.

1927.—A further note on *Nematospiroides dubius,* Baylis, 1926. Ann. and Mag. Nat. Hist., Ser. 9, 20:102–105.

1928.—On a collection of nematodes from Nigerian mammals (chiefly rodents). Parasit., 20:280–304.

Boulenger, C. L. 1922.—The structure and systematic position of *Strongylus polygyrus.* Parasit., 14:206–213.

Hall, M. C. 1916.—Nematode parasites of mammals of the orders Rodentia, Lagomorpha, and Hyracoidea. Proc. U. S. Nat. Mus., 50:1–258.

Lane, Clayton. 1923.—Some Strongylata. Parasit., 15:348–364.

Monnig, H. O. 1924.—South African parasitic nematodes. Ninth & Tenth Rep., Dir. Vet. Educ. and Research, Dept. of Agric., Union of South Africa.

1927.—On a new Physaloptera from an eagle and a Trichostrongyle from the Cane Rat, etc., Trans. Roy. Soc. S. Africa. 14:261–265.

Railliet, A., and Henry A. 1909.—Sur la classification des Strongylidae. I Metastrongylinae. C.r. Soc. biol., 66:85.

Roe, G. C. 1929.—A new nematode, *Sincosta aberrans,* new genus, and new species from a rodent. Proc. U. S. Nat. Mus., 75:1–3.

Schulz, R. E. 1926.—Zur Kenntnis der Helminthenfauna der Nagetiere der Union SSR. I. Strongylata, 1, Fam. Trichostrongylidae, Leiper., 1912. Trud. Ghosudarstv. Inst. Éksper. Veter., Moscow, 4:1–30.

Schulz, R. E., and Lubimov, M. P. 1932.—*Longistriata skrjabini* n. sp. (Nematoda, Trichostrongylidae) from the Ussuri squirrel. Parasit., 24:50–59.

Travassos, L. 1914.—Trichostrongylinae brazileiras. Braz. Med., 28:163, 186.

1918.—Trichostrongylidae brazileiras, Rev. Soc. brasil. Sciencias, 3:193.

1921.—Contribuçoes para o conhecimento da fauna helmintolojica brasileira. XIII. Ensaio monografico da familia Trichostrongylidae, Leiper 1909. Mem. Inst. Oswaldo Cruz, 13, 1:5–140.

Travassos, L., and Darriba, A. R. 1929.—Notas sobre Heligmosominae. Sci. Med., 7, No. 9.

Walton, A. C. 1923.—Some new and little known nematodes. Jour. Parasit., 10:59.

SOME NEW SPECIES OF TREMATODE WORMS OF THE GENUS *LEUCOCHLORIDIUM* CARUS, PARASITIC IN BIRDS FROM NORTHERN MICHIGAN, WITH A KEY AND NOTES ON OTHER SPECIES OF THE GENUS[1]

ALLEN MCINTOSH

Assistant Zoologist, Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C.

INTRODUCTION

During the summers of 1927, 1928, and 1929, post-mortem examinations for parasites were made, at the University of Michigan Biological Station, on 189 birds comprising 63 species. A total of 203 specimens of *Leucochloridium* were obtained from the cloaca and large intestine of 19 individuals, representing 11 species of birds. Seven species of *Leucochloridium* were found, 6 of which are described in this paper as new.

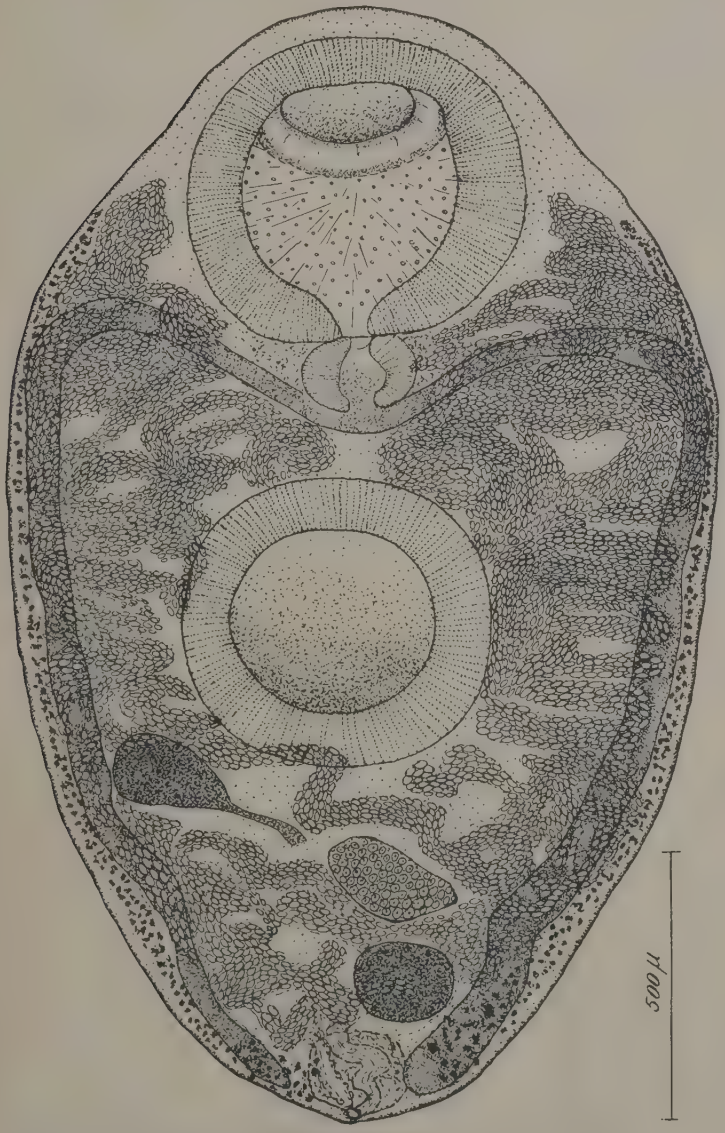
The writer wishes to express his appreciation to Doctor George R. LaRue, Director of the University of Michigan Biological Station, and to the other members of that Station, for privileges and courtesies extended to him while in residence there. The writer is also indebted to members of the Zoological Division of the Bureau of Animal Industry for aid in the preparation of the manuscript.

Leucochloridium cyanocittae n. sp.

Fig. 1.

Specific diagnosis: Body flat, ovate, 2.1 mm. long by 1.33 mm. wide in region between pharynx and acetabulum. Anterior sucker slightly subterminal, 560 μ by 630 μ. Pharynx 140 μ by 210 μ. Esophagus very short. Intestinal crura approximately uniform in diameter throughout entire length, left branch widest at posterior extremity; each branch extending into posterior end of body to area of cirrus pouch, the tips approximately 210 μ apart. Acetabulum somewhat smaller than oral sucker, 540 μ by 575 μ, situated near equator of body. Anterior testis with transverse diameter greater than longitudinal diameter, 130 μ by 200 μ; posterior testis 140 μ by 180 μ. Cirrus pouch about equal in size to posterior testis, 150 μ by 180 μ; cirrus 230 μ long, armed with spines. Genital pore at posterior end of body, median and terminal. Ovary 130 μ by 250 μ, situated anterior to posterior

[1] Contribution from Bureau of Animal Industry, U. S. Department of Agriculture, and the Biological Station of the University of Michigan.



Text Figure 1.—*Leucochloridium cyanocittae* n. sp., ventral aspect.

testis and occupying the same field; each testis and the ovary occupying separate zones. Fecundarium[2] indistinct. Vitellaria extracecal, extending from zone of cirrus pouch to posterior half of anterior sucker. Uterus filled with eggs, occupying all of intercecal space except area of acetabulum and area of cirrus sac, extending into extracecal area lateral to anterior sucker. Eggs 24 μ by 18 μ.

Type specimen.—U. S. Nat. Mus. Helm. Coll. No. 32397.

Host.—*Cyanocitta cristata bromia* (blue jay).

Location.—Cloaca.

Locality.—Michigan (Hook Point on Douglas Lake).

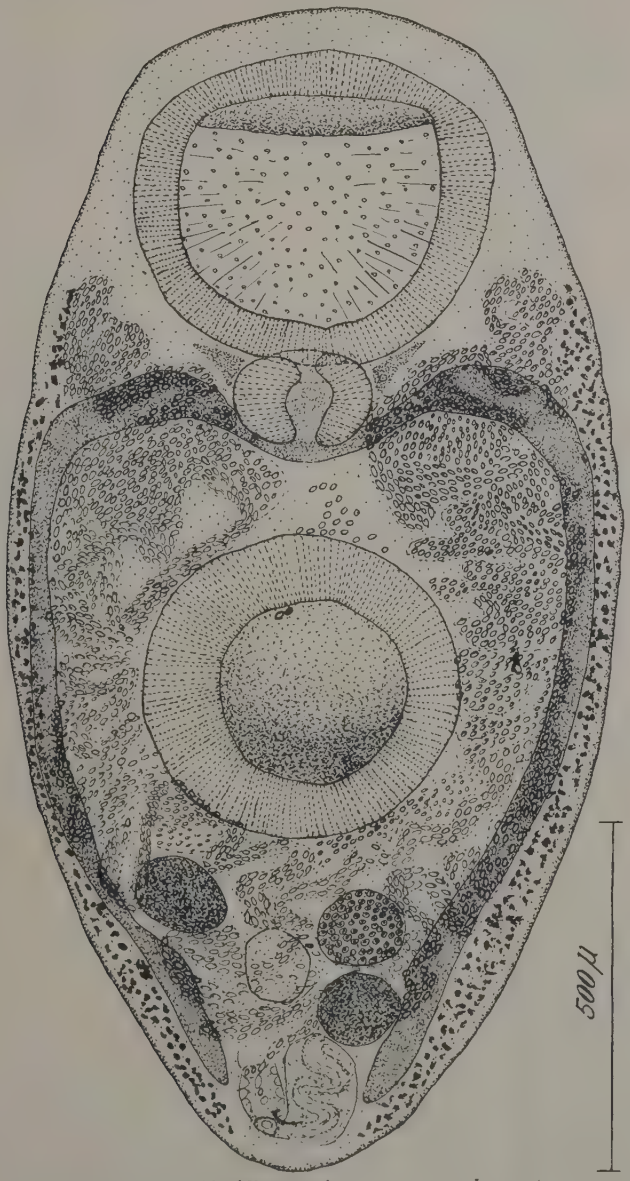
The above species has much in common with *L. actitis* n. sp. (p. 36) from the spotted sandpiper, *Actitis macularia.* In both species the vitellaria extend into the posterior part of the body, and the reproductive glands have the same general arrangement. Some of the outstanding differences between the two species are as follows: In *L. cyanocittae* the body, suckers, and pharynx are of greater size, and the anterior testis is well separated from the ovary, usually by a distance not less than the diameter of the testis; in *L. actitis* the ovary and anterior testis are closer together, and the pharynx is unusually small, the diameter being usually less than that of the ovary.

Leucochloridium variae n. sp.

Fig. 2.

Specific diagnosis: Body flat, ovate, 1.68 mm. long by 880 μ wide in front of acetabulum. Oral sucker 430 μ by 520 μ, subterminal. Pharynx broader than long, 140 μ by 190 μ. Esophagus very short. Intestinal crura extend into zone of cirrus pouch, the tips approximately 200 μ apart. Acetabulum approximating anterior sucker in size, 430 μ by 450 μ. Reproductive glands about equal in size; anterior testis 100 μ by 135 μ, adjacent to right branch of intestinal crura; posterior testis 100 μ by 115 μ, in contact with left branch of crura. Cirrus pouch 150 μ by 100 μ. Genital pore at posterior end of body, dorsal and subterminal. Ovary 100 μ by 120 μ, located on left side of body in posterior part of zone of anterior testis and directly in front of posterior testis. Fecundarium distinct, 100 μ by 95 μ, situated medial

[2] Some species of *Leucochloridium,* when stained *in toto,* show very distinctly a more or less spherical mass of cells near the ovary, having about the same staining properties as that organ. On sectioning, this mass of cells was found to be ova contained in a dilated portion of a tube leading from the ovary to Laurer's canal. This structure, designated as "ootype" in a previous paper (McIntosh, 1927), appears to be homologous with the structure commonly designated as the seminal receptacle by authors, and which, on account of its peculiar function as a fertilization chamber, has been given the name "fecundarium" by Sinitsin (1931).



Text Figure 2.—*Leucochloridium variae* n. sp., ventral aspect.

to interval separating ovary and posterior testis, and in line with testes. Vitellaria extracecal, extending from posterior level of cirrus pouch to zone of posterior fourth of anterior sucker. Uterus filled with eggs, confined to intercecal area with exception of short loop on each side of posterior part of anterior sucker. Eggs 25 μ by 16 μ.

Type specimen.—U. S. Nat. Mus. Helm. Coll. No. 32398.

Host.—Mniotilta varia (black-and-white warbler).

Location.—Cloaca.

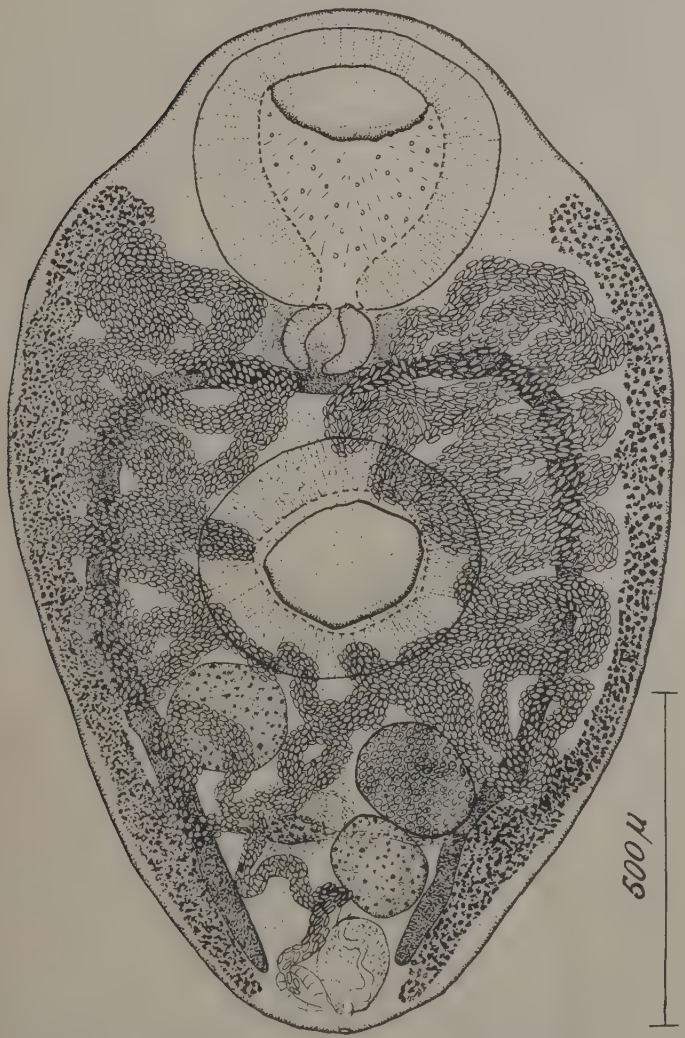
Locality.—Michigan (Hook Point on Douglas Lake).

The above species differs from *L. mniotiltae,* reported from the same host in Minnesota (McIntosh, 1927), in the general arrangement of the reproductive glands and in the extent of the vitellaria. The vitellaria in *L. mniotiltae* end at some distance anterior to the posterior extremities of the intestinal crura, while in *L. variae* they extend posteriorly beyond the tips of the crura. The arrangement of the reproductive glands in *L. variae* is similar to that in *L. cyanocittae,* but in *L. variae* the glands are much smaller and a well developed fecundarium is present. In *L. variae* the entire zone of the anterior testis is posterior to the zone of the acetabulum, while in *L. cyanocittae,* as well as in *L. mniotiltae,* the anterior testis is located partially in the posterior part of the acetabular zone.

Leucochloridium actitis n. sp.

Figs. 3 and 4.

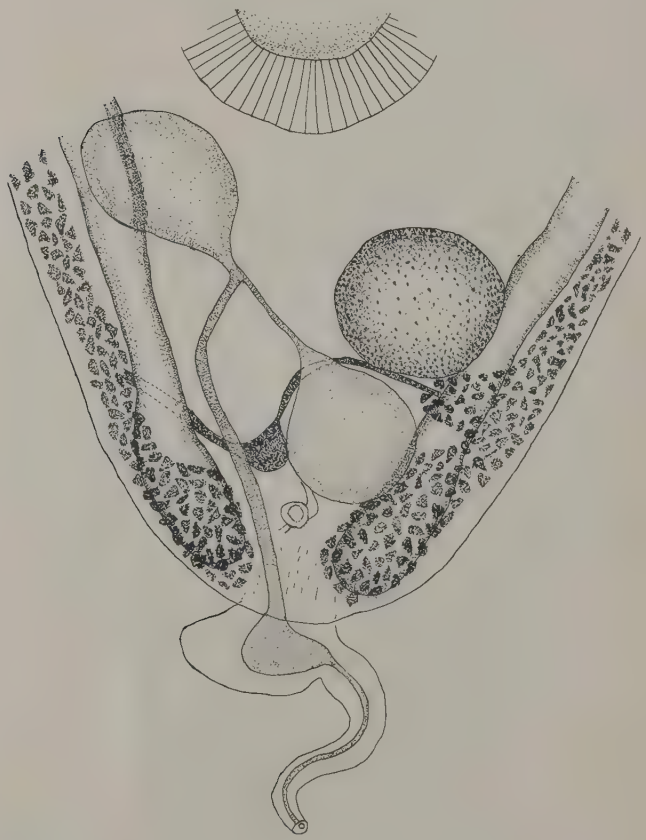
Specific diagnosis: Body flat, ovate, 1.72 mm. long by 960 μ wide in front of acetabulum. Oral sucker 400 μ by 400 μ, subterminal. Pharynx 100 μ by 150 μ. Esophagus very short. Intestinal crura extending posteriorly into zone of cirrus pouch, the tips approximately 70 μ apart. Acetabulum 370 μ by 400 μ, near center of body. Reproductive glands almost spherical; anterior testis 175 μ by 175 μ, located to left of median line of body and immediately posterior to acetabulum; posterior testis 170 μ by 150 μ, situated to right of median line and immediately in front of posterior extremity of right branch of intestinal crura. Cirrus pouch approximately spherical, measuring 120 μ in diameter in specimens with cirrus not extended; cirrus 300 μ long when protruded, armed with spines. Genital pore at posterior end of body, median and terminal. Ovary 160 μ by 170 μ, diagonally in front of posterior testis and adjacent to right branch of intestinal crura, occupying a part of the field of the posterior testis and a part of the zone of the anterior testis; zones of ovary and posterior testis contiguous. Fecundarium indistinct. Vitellaria extracecal, extending from posterior extremities of intestinal crura to zone of posterior fourth of anterior sucker. Uterus filled with eggs, occupying most



Text Figure 3.—*Leucochloridium actitis* n. sp., ventral aspect.

of intercecal area and extending into antero-lateral extracecal area. Eggs 22.5 μ by 17 μ.

Type specimen.—U. S. Nat. Mus. Helm. Coll. No. 32399.
Host.—*Actitis macularia* (spotted sandpiper).
Location.—Cloaca.
Locality.—Michigan (Cecil Bay near Douglas Lake).



Text Figure 4.—*Leucochloridium actitis* n. sp., posterior extremity of body showing male reproductive system.

In some specimens of the above species the position of the reproductive glands was observed to be reversed; that is, the anterior testis occurs on the right instead of the left side of the body, with the ovary and posterior testis on the opposite side. Individuals of this species

reach sexual maturity early and before attaining maximum size. Specimens as small as 1 mm. long, taken from juvenile birds, contained numerous eggs. This species, as pointed out above, has many points in common with *L. cyanocittae*. In comparing the two species it is well to bear in mind that *L. actitis* has a comparatively small pharynx, the diameter of which seldom exceeds that of the ovary, while in *L. cyanocittae* the pharynx is usually larger than the ovary.

Witenberg (1925) described and figured a fluke from a closely related host, *Actitis hypoleuca,* under the name *L. insigne* (Looss), which, in the opinion of the writer, is not *L. insigne* (Looss), but is a species closely related to, if not identical with, the form described here as *L. actitis*. A comparison of Witenberg's figures 10–13 with figures 3 and 4 of this paper shows the similarity of these two forms from related hosts. The writer separates *L. actitis* from *L. insigne* (Looss) largely on the basis of the vitellaria which extend into the area lateral to the oral sucker in *L. actitis,* while in *L. insigne* (Looss) the vitellaria end at the level of the pharynx. *L. insigne* (Looss) is also much larger, and was taken from an unrelated host, the European coot, *Fulica atra.*

Leucochloridium melospizae n. sp.

Fig. 5.

Specific diagnosis: Body flat, ovate, 1.41 mm. long by 920 μ wide. Oral sucker 400 μ by 500 μ, subterminal. Pharynx 120 μ by 175 μ. Esophagus very short. Intestinal crura end somewhat posterior to zone of posterior testis, and 200 μ from posterior end of body, the tips 170 μ apart. Acetabulum circular, 420 μ in diameter, located about at center of body and immediately posterior to pharynx. Reproductive glands comparatively small; anterior testis 70 μ by 80 μ, adjacent to right branch of intestine, and slightly posterior to zone marking posterior level of acetabulum; posterior testis more or less spherical, slightly larger than anterior testis, 100 μ in diameter, located anterior and median to posterior extremity of left branch of intestine. Cirrus pouch 100 μ by 120 μ; a well developed seminal vesicle present, 90 μ in diameter; cirrus, when protruded, about 120 μ long. Genital pore at posterior end of body, slightly subterminal. Ovary 80 μ by 100 μ, immediately anterior to posterior testis, occupying zone of anterior testis and field of posterior testis. Fecundarium prominent, about size of anterior testis, located between the testes and in line with them, and opposite the interval of ovary and posterior testis. Vitellaria extracecal, beginning anteriorly at level of posterior third of oral sucker and terminating posteriorly opposite the level of tips of intestinal crura. Uterus with numerous eggs, confined mostly to intercecal space, but a

Text Figure 5.—*Leucochloridium melospizae* n. sp., ventral aspect.

few convolutions extend over ventral surface of intestine both laterally and anteriorly. Eggs 22 μ by 17 μ.

Type specimen—U. S. Nat. Mus. Helm. Coll. No. 32400.

Host.—Melospiza melodia beata (song sparrow).

Location.—Cloaca.

Locality.—Michigan (Monro Lake near Douglas Lake).

The above species resembles more closely *L. macrostomum* as figured by Zeller (1874) than any of the American species of the genus thus far described. In both species the vitellaria and intestinal crura end at the same level and at some distance before reaching the posterior end of the body. One of the outstanding differences is the small size of the reproductive glands in *L. melospizae*, the diameters of which are scarcely more than one-half that of the pharynx. The arrangement of the sex glands, including the fecundarium, in *L. melospizae* is also similar to the arrangement in *L. variae*, but the two species may be separated by the extension of the intestinal crura and the vitellaria, which in *L. variae* end in the zone of the cirrus pouch.

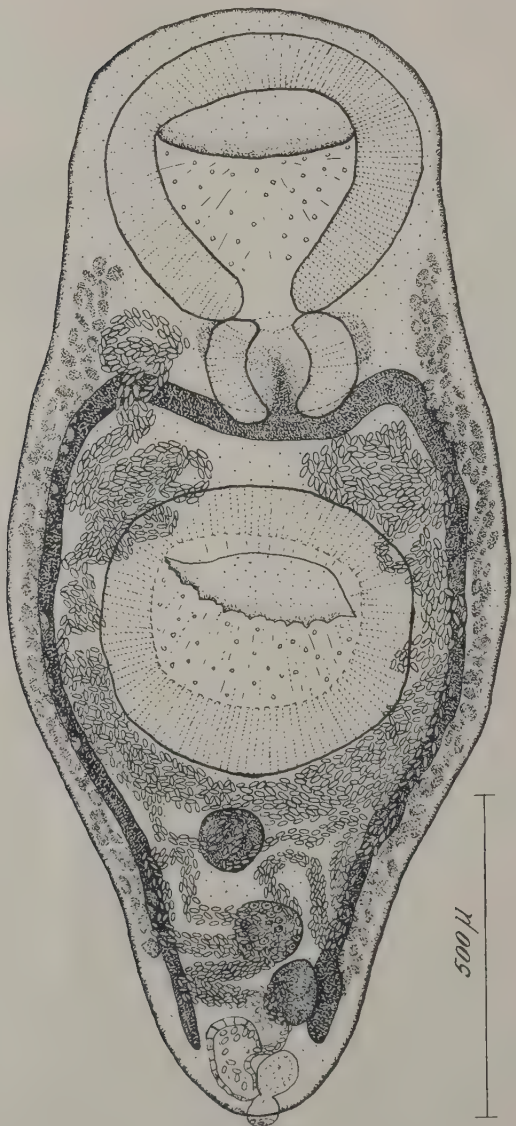
Leucochloridium seiuri n. sp.

Fig. 6.

Specific diagnosis: Body 1.73 mm. long by 750 μ wide, broadest at acetabulum, posterior part narrower and more attenuated than anterior half. Oral sucker 450 μ by 470 μ, subterminal. Pharynx 210 μ by 220 μ. Esophagus very short. Intestinal crura end near zone of cirrus pouch, the tips 160 μ apart. Acetabulum 420 μ by 440 μ, slightly post-equatorial. Reproductive glands about equal in size, arranged in a linear series diagonal to longitudinal axis of body; anterior testis 80 μ by 90 μ, located slightly to right of median line and less than half its diameter from acetabulum; posterior testis 100 μ by 70 μ, located near posterior extremity of left branch of intestinal crura; testicular fields contiguous. Cirrus pouch 50 μ in diameter; cirrus short and bulbous, measuring, when protruded, 40 μ long by 54 μ wide. Genital pore at posterior end, mediodorsal and subterminal. Ovary 80 μ by 90 μ, situated partly in field of both testes with its zone contiguous with zone of posterior testis. Fecundarium indistinct. Vitellaria end posteriorly at level of posterior testis, and anteriorly at zone of posterior portion of oral sucker. Uterus filled with numerous eggs, confined largely to intercecal space but sometimes sending a loop forward on one side into extracecal area as far as oral sucker. Metraterm well developed. Eggs 23 μ by 18 μ.

Type specimen.—U. S. Nat. Mus. Helm. Coll. No. 32401.

Host.—Seiurus aurocapillus aurocapillus (oven bird).



Text Figure 6.—*Leucochloridium seiuri* n. sp., ventral aspect.

Location.—Cloaca.

Locality.—Michigan (Monro Lake near Douglas Lake).

The above species, with the decidedly tapering posterior end, the linear arrangement of the reproductive glands, and with no anterior extension of the uterus into the area lateral to the oral sucker, is distinctly different from the other species of the genus *Leucochloridium.*

Leucochloridium dryobatae n. sp.

Fig. 7.

Specific diagnosis: Body 2.19 mm. long by 1.28 mm. wide, flat, ovate, with greatest width across anterior margin of acetabulum. Oral sucker 480 μ by 560 μ, slightly subterminal. Pharynx 170 μ by 250 μ. Esophagus very short. Intestinal crura terminate a short distance beyond posterior testis, the tips 270 μ apart. Acetabulum 570 μ by 620 μ, slightly post-equatorial. Reproductive glands arranged in a linear series; anterior testis 160 μ by 200 μ, situated near median line of body, and about half its diameter from acetabulum; posterior testis 140 μ by 190 μ,situated to left of median line of body and in contact with posterior extremity of left branch of intestinal crura. Cirrus pouch small, 60 μ in diameter; cirrus short. Genital pore at posterior end of body, median and terminal. Ovary 170 μ by 220 μ, in line with testes. Each reproductive gland occupies a separate zone but their fields coincide, at least in part. Fecundarium indistinct. Vitellaria extracecal, ending posteriorly at about level of posterior zone of ovary, and anteriorly at level of posterior fourth of oral sucker. Metraterm muscular, situated to right of median line of body. Uterus filled with eggs, occupying all available intercecal space and extending into and filling area lateral to pharynx and oral sucker. Eggs 25 μ by 18 μ.

Type specimen.—U. S. Nat. Mus. Helm. Coll. No. 32402.

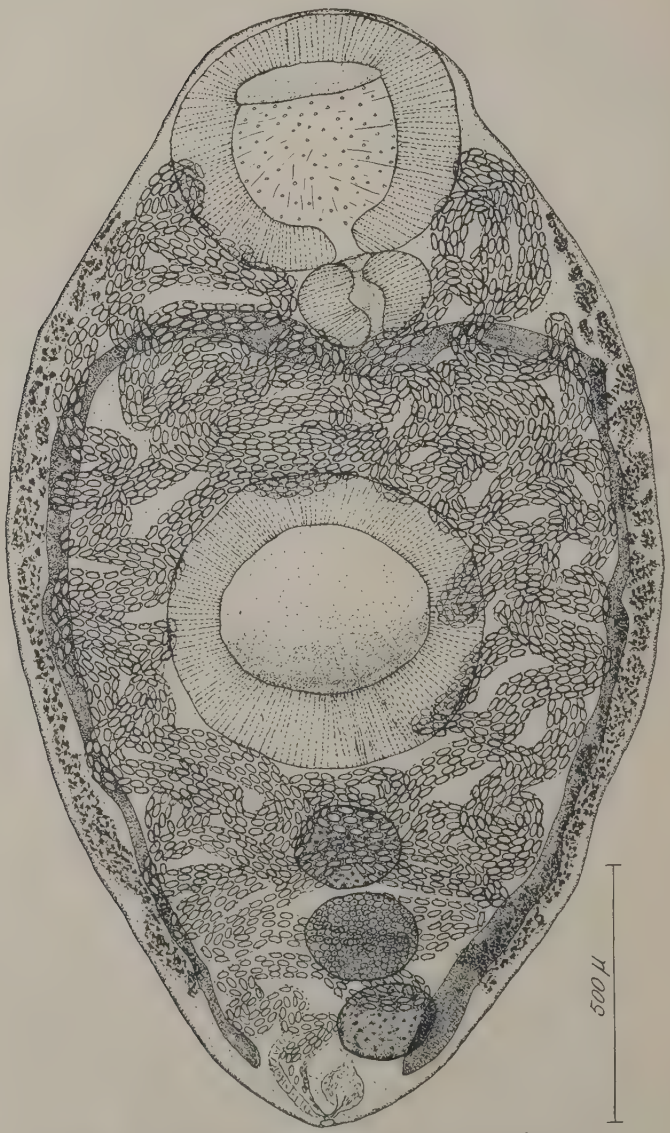
Host.—Dryobates villosus villosus (hairy woodpecker).

Location.—Cloaca.

Locality.—Michigan (Hook Point on Douglas Lake).

In the above species the reproductive glands are arranged in linear series similar to the arrangement found in *L. seiuri,* which is a much smaller form. But, aside from the difference in size and shape, the two species may be readily separated by the position of the uterus, which in *L. seiuri* is largely confined to the intercecal area, while in *L. dryobatae* it extends into the area lateral to the anterior sucker.

In addition to the type host, hairy woodpecker, *Dryobates villosus villosus,* the following species of birds taken at Douglas Lake, Michigan, harbored flukes which are at present identified as *L. dryobatae:* Yellow-bellied sapsucker, *Sphyrapicus varius varius;* towhee, *Pipilo erythrophthalmus erythrophthalmus;* scarlet tanager, *Piranga erythro-*



Text Figure 7.—*Leucochloridium dryobatae* n. sp., ventral aspect.

melas; red-eyed vireo, *Vireosylva olivacea;* cowbird, *Molothrus ater ater;* and song sparrow, *Melospiza melodia beata.* There is some variation in the arrangement of the sex glands in the specimens from the different hosts, but the variation does not appear to be greater than that existing in a series of individuals from a single host.

Leucochloridium certhiae McIntosh, 1927.

Fig. 8.

Leucochloridium certhiae was described from the brown creeper, *Certhia familiaris americana,* from Minnesota in 1927 by the writer. This species is characterized by unusually large testes, the diameters of which are about twice the diameter of the ovary. In Michigan this parasite was not taken from the brown creeper, of which only a single specimen was examined, but was found in the song sparrow, *Melospiza melodia beata.* The specimens from the song sparrow differ only in minute detail from the type specimen.

Witenberg (1925) described a trematode from *Muscicapa grisola–M. striata* which he called *Leucochloridium* sp. This form is also characterized by large testes which almost fill the region of the body posterior to the acetabulum. There is no doubt but that this Old World species is closely related to, if not identical with, *L. certhiae.*

Type specimen.—U. S. Nat. Mus. Helm. Coll. No. 32392.

Hosts.—Certhia familiaris americana (brown creeper); *Melospiza melodia beata* (song sparrow); and (?) *Muscicapa striata* (spotted flycatcher).

Location.—Cloaca.

Localities.—Minnesota (St. Paul); Michigan (Monro Lake); and (?) Tschardschuj (in Buchara).

OTHER AMERICAN SPECIES

The following are the species of *Leucochloridium,* other than those described here, that have been reported from North American birds. Some of these will be found in time, no doubt, in Michigan birds.

Leucochloridium sorae McIntosh, 1927.

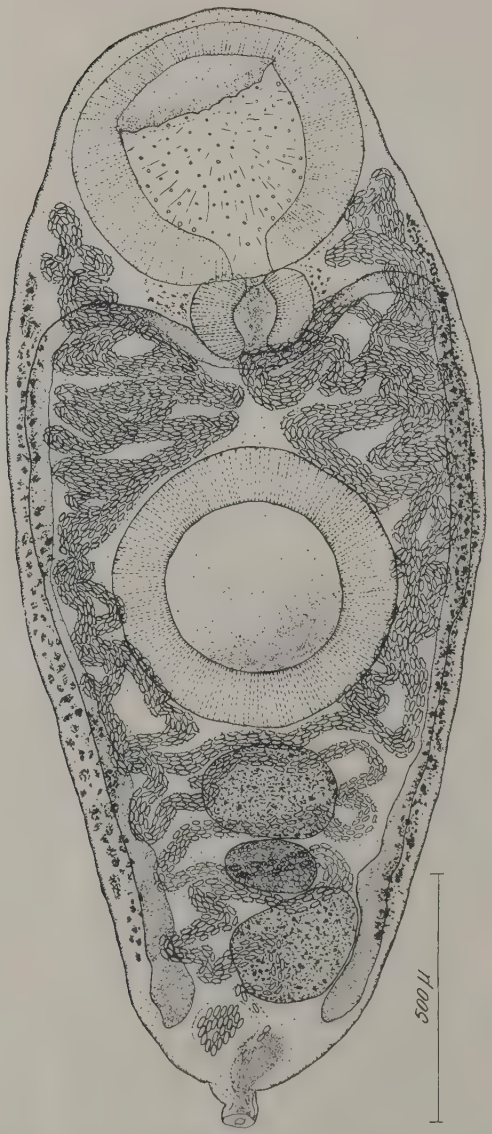
This species is characterized by a pre-equatorial acetabulum, the uterus confined to intercecal area, and the posterior testis situated at a considerable distance from posterior extremities of vitellaria and intestinal crura.

Type specimen.—U. S. Nat. Mus. Helm. Coll. No. 32396.

Host.—Porzana carolina (sora rail).

Location.—Intestine.

Locality.—Minnesota (Rice Creek, Minneapolis).



Text Figure 8.—*Leucochloridium certhiae* McIntosh, 1927, ventral aspect.

Leucochloridium mniotiltae McIntosh, 1927.

This is a small species, characterized by vitellaria not extending posteriorly as far as the intestinal crura, testes and ovary arranged in a linear series, a well developed fecundarium, and the uterus confined to post-oral area.

Type specimen.—U. S. Nat. Mus. Helm. Coll. No. 32393.
Host.—*Mniotilta varia* (black-and-white warbler).
Location.—Large intestine.
Locality.—Minnesota (University Farm, St. Paul).

Leucochloridium icteri McIntosh, 1927.

This species is characterized by the triangular arrangement of the reproductive glands, and by the vitellaria ending anteriorly in zone of oral sucker and terminating posteriorly in zone of posterior testis.

Type specimen.—U. S. Nat. Mus. Helm. Coll. No. 32394.
Host.—*Icterus galbula* (Baltimore oriole).
Location.—Rectum.
Locality.—Minnesota (University Farm, St. Paul).

Leucochloridium vireonis McIntosh, 1927.

A comparatively large species, characterized by pre-equatorial acetabulum, vitellaria terminating posteriorly near zone of anterior testis, and by reproductive glands situated nearer to posterior end of body than to acetabulum.

Type specimen.—U. S. Nat. Mus. Helm. Coll. No. 32395.
Host.—*Vireo griseus griseus* (white-eyed vireo).
Location.—Large intestine.
Locality.—Mississippi (A. and M. College).

INCIDENCE AND DEGREE OF PARASITISM

In Table 1 there are included, as a matter of record, data which may be of value.

As mentioned in the introduction, post-mortem examinations were made of 189 birds comprising 63 species. In Table 1 only the species found harboring *Leucochloridium* are given. Many of the individuals that had *Leucochloridium* also harbored other species of distomes.

Notwithstanding the fact that in most cases the random sampling has been of too few individuals to warrant definite conclusions regarding the extent of parasitism, there are a number of interesting facts to be noted. Of the 57 specimens of birds listed in the table, the ratio of the infested adults to the uninfested adults is 12 : 27, or 30.76 per cent

TABLE 1.—*Data on Incidence and Degree of Parasitism with Leucochloridium in Eleven Species of Birds from North Michigan.*

Host	Birds examined		Birds with Leucochloridium		Birds Negative		No. of Leucochloridium from—		Remarks concerning parasites
	Adults	Juveniles	Adults	Juveniles	Adults	Juveniles	Adults	Juveniles	
Mniotilta varia	3	–	1	–	2	–	3	–	All adults.
Cyanocitta cristata bromia	4	1	2	–	2	1	21 1	–	All adults.
Molothrus ater ater	–	4	–	1	–	3	–	7	All adults.
Dryobates villosus villosus	1	–	1	–	–	–	1	–	Adult, very large.
Seiurus aurocapillus aurocapillus	2	–	1	–	1	–	28	–	3 immature, 25 adults.
Vireosylva olivacea	8	–	1	–	7	–	8	–	All adults.
Piranga erythromelas	2	–	1	–	1	–	12	–	All adults.
Melospiza melodia beata	5	3	2	2	3	1	3 2	10 8	Adults, several immature forms not counted.
Actitis macularia	8	8	1	3	7	5	2	1 4 7	12 adults, many immature forms not counted.
Pipilo erythrophthalmus erythrophthalmus	4	2	1	1	3	1	1	68	All mature.*
Sphyrapicus varius varius	2	–	1	–	1	–	16	–	All adults.
Total	39 + 57	18	12 + 19	7	27 + 38	11	98 + 203	105	

* Any fluke containing eggs is considered sexually mature.

parasitized, while the ratio of the infested juvenile birds to the uninfested juvenile birds is 7 : 11, or 38.88 per cent parasitized. These figures, although based on too few records, indicate that more young birds than adults are parasitized.

It is also interesting to note that the parasitized juvenile birds, which represent 12.28 per cent of all the birds examined, yielded 51.72 per cent of the gross number of parasites collected. In contrast to this, the parasitized adults, which represent 21.05 per cent of the birds, yielded 48.28 per cent of the parasites collected. These results indicate that the juvenile birds harbor a greater number of parasites per bird than do the adults.

Since parasitism is heavier and more prevalent in young birds than in adults, the question arises as to how this difference in degree of parasitism can be explained. Is an immunity developed on the part of the adult, or is this difference due largely to the difference in the type of diet between the juvenile and adult forms? The latter might be an explanation for such birds as sparrows, which feed largely on seeds and fruit in the adult stage, while the young are fed mostly on animal matter, such as insects, snails, etc., and are thus afforded a better opportunity to become parasitized. In spite of the meager data given, there is some evidence to support the immunity theory. In all cases, except one, where both adults and young birds of the same species were examined, the juvenile birds constituted the higher percentage of the parasitized birds, and also harbored the larger number of individual parasites per bird. An interesting case is that of the spotted sandpiper, *Actitis macularia*, in which 16 individuals were examined, 8 of which were adult birds. Of the 8 adults, 7 were negative, and the infested bird had only 2 specimens of *Leucochloridium*, both of which were immature; of the 8 juvenile birds, 3 had parasites of which several were adults and many were immature forms.

UNUSUAL HOST RECORDS

The finding of trematode parasites in two species of the family *Picidae* is of unusual interest. There are very few records of members of this family harboring trematodes. Likewise the record from the song sparrow, *Melospiza melodia beata*, is of interest, because sparrows are comparatively free from flukes, as a rule, due to their seed-eating habits.

ENZOOTIC AREA

A thorough study of the land snails in the Douglas Lake area will, no doubt, reveal that they harbor the immature stages of the adult species of *Leucochloridium* found in the birds of that area. The land

snails are abundant in number of species as well as in number of individuals. There are in the Douglas Lake area at least three species of *Succinea,* a genus of snails known to harbor the immature stages of *Leucochloridium.* One of the species, *Succinea ovalis,* is found in wooded areas, the habitat of such birds as the oven bird, towhee, and scarlet tanager. The other two species of *Succinea* are found on the lake shore, often near the edge of the water, which is the habitat of the spotted sandpiper, a bird that is seldom seen elsewhere. In spite of the apparent abundance of adult *Leucochloridium* in birds, few naturalists have observed the colorful sporocysts of the immature stage in the snails of that area. The writer has collected and examined hundreds of individuals in search of the classical multi-colored sporocyst that is supposed to be found in the tentacle of the snail, but has not as yet found any parasitized forms.

AN ALASKAN LEUCOCHLORIDIUM

After having completed this manuscript, there was placed at the writer's disposal, by Dr. E. W. Price, of the Zoological Division of the Bureau of Animal Industry, 8 specimens of *Leucochloridium* recently received from Alaska. This material was collected by Mr. A. H. Twichell from *Canachites canadensis osgoodi* (Alaska spruce grouse or spruce patridge), and constitutes the only record thus far of *Leucochloridium* from Alaska.

Since the spruce grouse is a non-migratory bird, the immature stages of this parasite most probably occur in land snails of the same area.

This material represents a new species, as described below.

Leucochloridium pricei n. sp.

Fig. 9.

Specific diagnosis: Body ovate, 1.17 mm. long by 540 μ wide in region of acetabulum. Anterior sucker slightly subterminal, 360 μ by 360 μ. Pharynx 120 μ by 140 μ. Esophagus very short. Intestinal crura extend into posterior end of body to area of cirrus pouch, the tips approximately 140 μ apart. Acetabulum 340 μ by 360 μ, situated near equator of body. Anterior testis transversely oval, 80 μ by 130 μ; posterior testis 80 μ by 105 μ. Cirrus pouch 140 μ by 80 μ; cirrus short, globular. Genital pore at posterior end of body, dorsal and subterminal. Ovary spherical, 60 μ in diameter, situated near median line of body directly in front of posterior testis and occupying a part of the zone of the anterior testis. Fecundarium indistinct. Vitellaria extracecal, beginning near the level of the posterior margin of the anterior sucker and ending at or near the tips of the intestinal crura.

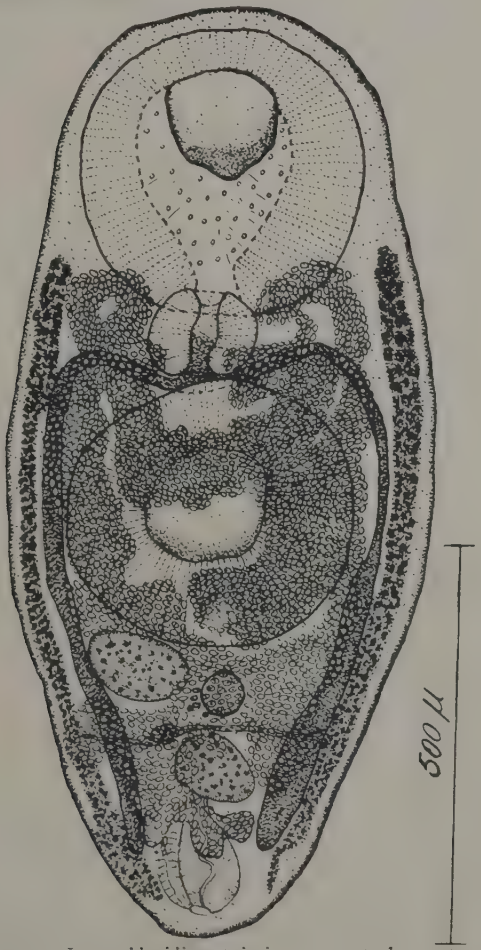
Uterus filled with eggs, occupying most of intercecal area and area lateral to the pharynx. Eggs 23 μ by 17 μ.

Type specimen.—U. S. Nat. Mus. Helm. Coll. No. 32404.

Host.—*Canachites canadensis osgoodi* (Alaska spruce grouse).

Location.—Intestine.

Locality.—Alaska.



Text Figure 9.—*Leucochloridium pricei* n. sp. ventral aspect.

The above species with the reproductive glands arranged in a triangle, and with the very small ovary situated near the median line

of the body, is regarded as distinct from the other North American species.

The following key will aid in distinguishing the known species of *Leucochloridium*:

1. Acetabulum pre-equatorial; uterus confined to intercecal area; vitellaria extending posteriorly beyond level of posterior testis 2
   The above combination of characters not present 5
2. Over 5 mm. long; testes lobed ..... *L. flavum* Trav.
   Less than 5 mm. long; testes oval or spherical 3
3. Ovary post-testicular ..... *L. cercatum* (Mont.)
   Ovary anterior to zone of posterior testis 4
4. Genital pore ventral; ovary and testes spherical; ovary occupying a part of zone of anterior testis ..... *L. turanicum* (Sol.)
   Genital pore dorsal; ovary and testes elliptical; ovary and anterior testis occupying separate zones[3] ..... *L. sorae* McI.
5. Ovary and testes arranged in a triangle 6
   Ovary and testes arranged more or less in a linear series 16
6. Diameter of ovary about one-half that of testes 7
   Diameter of ovary about equal to that of testes or larger 8
7. Vitellaria not extending posteriorly beyond zone of ovary .. *L. parcum* Trav.
   Vitellaria extending posteriorly beyond zone of ovary, usually to tip of cecum or beyond ..... *L. pricei* n. sp.
8. Vitellaria extending well beyond zone of posterior testis into posterior tip of body to area of cirrus pouch 9
   Vitellaria not extending to tip of body, usually ending at or before reaching zone of posterior testis 12
9. Vitellaria extending anteriorly only to level of pharynx; uterus circling acetabulum and not extending anteriorly beyond bifurcation of intestine ..... *L. insigne* (Looss)
   Vitellaria extending anteriorly beyond level of pharynx; uterus not circling acetabulum but extending on each side of body anteriorly beyond bifurcation of intestine 10
10. Anterior testis and ovary separated by a distance equal to, or less than, longitudinal diameter of either of these organs; ovary and anterior testis with their zones partly overlapping or entirely so; transverse diameter of pharynx usually less than longitudinal diameter of ovary .... *L. actitis* n. sp.
    Anterior testis and ovary separated by a distance equal to, or greater than, longitudinal diameter of either of these organs; transverse diameter of pharynx greater than longitudinal diameter of ovary 11
11. Fecundarium conspicuous; ovary and pharynx with a transverse diameter ratio of 5 : 8 ..... *L. variae* n. sp.
    Fecundarium inconspicuous; ovary and pharynx ratio about 5 : 6 *L. cyanocittae* n. sp.
12. Vitellaria extending posteriorly as far as tip of intestine 13
    Vitellaria not extending posteriorly as far as tip of intestine 14
13. Fecundarium well developed ..... *L. melospizae* n. sp.
    Fecundarium indistinct ..... *L. macrostomum* (Rud.)
14. Distance separating posterior testis from tip of body much less than distance separating anterior testis from acetabulum .... *L. vireonis* McI.
    Distance separating posterior testis from tip of body about equal to, or greater than, distance separating anterior testis from acetabulum 15

[3] *L. hypotaenidiarum* Tubangui, 1932, will key out with *L. sorae;* the two species are very similar, as Tubangui (1932) has pointed out.

15. Vitellaria extending for some distance anteriorly beyond bifurcation of intestine along each side of oral sucker .....*L. icteri* McI.
Vitellaria not extending anteriorly beyond level of bifurcation of intestine .....*L. dasylophi* Tub.
16. Testes large, about twice diameter of ovary .....*L. certhiae* McI.
Testes with a diameter about equal to, or less than, that of ovary 17
17. Uterus extending anteriorly into the area lateral to oral sucker *L. dryobatae* n. sp.
Uterus not extending anteriorly into the area lateral to oral sucker 18
18. Anterior testis overlapped ventrally on its anterior margin by acetabulum; fecundarium well developed .....*L. mniotiltae* McI.
Anterior testis usually posterior to zone of acetabulum; fecundarium not developed .....*L. seiuri* n. sp.

In the key, as well as in the descriptions of the species, the writer has used extensively the distribution of the vitellaria and the arrangement of the reproductive glands in separating and characterizing the different species. The position and the arrangement of the reproductive glands may, and doubtless do, often vary in the different individuals of a species. Such variations of the reproductive glands, due to body contractions or possible disarrangement by the gravid coils of the uterus, are confusing and may lead to error in separating species. In the majority of cases, however, where a large number of specimens were taken from a single host, the individuals of a single species were found to be fairly uniform and similar in general appearance.

References

McIntosh, A. 1927.—Notes on the genus *Leucochloridium* Carus (Trematoda). Parasitology, 19:353–364.

Sinitsin, D. 1931.—Studien über die Phylogenie der Trematoden. V. Revision of Harmostominae in the light of new facts from their morphology and life history. Ztschr. Parasitenk., 3:786–835.

Tubangui, M. A. 1932.—Trematode parasites of Philippine vertebrates, V. Flukes from birds. Philippine J. Sc. 47:369–404.

Witenberg, G. 1925.—Versuch einer Monographie der Trematodenunterfamilie Harmostominae Braun. Zool. Jahrb., Jena, Abt. f. Syst., 51:167–254.

Zeller, E. 1874.—Ueber *Leucochloridium paradoxum* Carus und die weitere Entwickelung seiner Distomenbrut. Zeitschr. Wiss. Zool., 24: 564–578.

NOTE ON RE-INFECTION UNDER "NATURAL" CONDITIONS WITH A GUT NEMATODE OF THE RABBIT*

Norman R. Stoll

The occurrence in domestic sheep of self-cure and protection against the common stomach worm, *Haemonchus contortus,* under conditions of natural re-infection (Stoll, 1929), led to tests of the same possibility with other worm-host systems. The rabbit appeared to offer the advantage of a useful small-sized host for such study, and wild rabbits of the region as well as some of the domestic stock in the laboratory carried low-grade infections with the small strongyloid *Trichostrongylus calcaratus,* which inhabits the jejunum. This nematode has the characteristic life history of worms of this group: males and females more or less localized during their adult life in a relatively small area of the food tract; no multiplication of the parasites *in vivo* but the extrusion of eggs into the lumen of the tract from whence they escape to the outer world in the feces; then the development in the dung of larval stages, which after an interval of a week or so at summer temperatures become infective, re-enter the host either through the food or skin, and assume final development to adult life within about two weeks in the gut area earlier parasitized by the adult worms which produced them. Such a cycle is characteristic of *H. contortus,* as it is of the hookworms and allied forms.

It seemed obvious that if rabbit hosts could be kept under conditions where they would come in contact with soil upon which infected feces would permit larval stages to develop, re-infection would take place, the hosts would be assaulted by gradually increasing numbers of worms and be forced to exhibit their capacity to cope with the parasites.

The requirements of such an experiment appear to be so simple as to make its repetition with various worm-host systems almost an elementary procedure. Experience has taught that practical problems involved in the experimentation frequently render it difficult to resolve however. An early experiment with *T. calcaratus* in domestic rabbits giving results of enough interest to cause its further exploration in some detailed experiments indoors (Sarles, 1932) warrants this preliminary note.

In brief, these results show:

First, that the theoretical demands of the experiment can be met with this worm and host, namely, that clean rabbits will pick up the

* From the Department of Animal and Plant Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.

infection from an area upon which feces containing the eggs of this parasite are deposited, and that reinfection, as judged by egg counts, will take place; and

Second, that while the response of individual hosts is variable, the infection may reach a relatively high level followed in some cases by an indication of a resistance and resultant loss of worms.

These two results are the first such demonstration with this host and parasite.

EXPERIMENTAL

Rabbits from the laboratory stock were utilized. As this stock, particularly at certain seasons, had been known to harbor *T. calcaratus*, precautions were taken to secure animals free from infection as gauged by careful examination of feces by a flotation technique (Stoll, 1930). All these rabbits had coccidia and a few were lightly infected with an oxyurid, *Passalurus ambiguus*. Neither of these parasites appear to have impaired significantly the health of the hosts or the results with Trichostrongylus however.

Preliminary experiments with *T. calcaratus* showed that infection by filariform larvae was possible through both mouth and skin.

Larvae grown in stock cultures of rabbit dung, and isolated by the Baermann apparatus, were used in the following illustrative tests. Rabbit 11, age three months, with feces negative in 5 examinations over an 11 day period, was fed 20 *T. calcaratus* filariform larvae by mouth on May 17, 1928. Five examinations in the next 14 days were negative for ova. On the 16th day there were 26 *T. calcaratus* eggs per gram of dung, 32 per gram on the 19th day, and 6, 12 and 10 per gram on the 25th, 32nd and 39th days after infection. This animal was later used to begin the re-infection experiment.

Rabbit 12, two months old, was used for skin infection. On May 17th, 40 filariform larvae in water were placed on the unabraded skin of the back, after a preliminary wetting of the hair. At 6, 8 and 14 days after infection the animal's feces continued negative; at 16 days 18 *T. calcaratus* eggs per gram were demonstrable. Counts on the 19th and 25th days were respectively 12 and 6 per gram, on the 32nd and 39th days, negative. On the 40th day an additional 15 infective larvae were placed on the unabraded, wet skin of the back. The next examination at 69 days showed a count of 4 *T. calcaratus* eggs per gram, and in 10 examinations about equally spaced over 77 additional days, the animal, kept in a cage designed and believed to exclude re-infection, continued positive averaging 11 eggs per gram (range 4 to 20). On October 12th the animal was killed and 3 adult Trichostrongylus, all females, were recovered.

These two cases are sufficient to show that infections by both mouth and skin are demonstrable, and that even light infections can be shown by the flotation examination of the feces.

The approximate degree of infection with strongyloid worms inhabiting the mammalian gastro-enteric tract is measurable by the number of eggs extruded by way of the feces. This correlation, while not perfect, is sufficiently serviceable to show significant differences in infection levels. The flotation technique used on sheep feces for Haemonchus eggs is highly efficient (Stoll, 1930) routine expectation being that each successive cover after centrifugation from the tube, will remove about nine-tenths of the eggs in the tube. With *T. calcaratus* eggs in rabbit dung each cover removed only about two-thirds of the eggs present. In practice this meant that more covers from a given tube were examined to get adequate counts from rabbit dung than from sheep.

For the experiment itself, the requirement was to place a group of rabbits on soil in a wire-enclosed area from which they could not escape, with one animal infected with *T. calcaratus,* and determine to what degree they would all pick up infection from the area which would be gradually "seeded" by the one infected animal; and thus to determine whether host response to increasing worm increments would be continually in favor of the parasites or of the hosts. This is a type of epidemiological experiment in which all "susceptibles" are equally exposed throughout. The soil enclosure used was 5×20 feet, with a cement-floored room into which the rabbits came for food and drink, and for housing at night. The soil area itself, their runway, was clay-loam without vegetation, exposed to both sun and rain, and was occasionally raked up on the surface but not otherwise disturbed. It was shaded in the afternoons. Dung passed in the cement floored room was swept back onto the soil area daily. Appropriate care was taken that other animals did not have contact with the experimental group, although sparrows were occasionally noted in the area. The rabbits were fed fresh alfalfa hay and grain.

Fecal collections from the animals were obtained by placing them in individual screen-bottom cages for 24 hours each week, during which time they were fed on cabbage leaves, the residue of which could be easily removed. During the experimental period, June to November, only pellet type feces were passed by the animals.

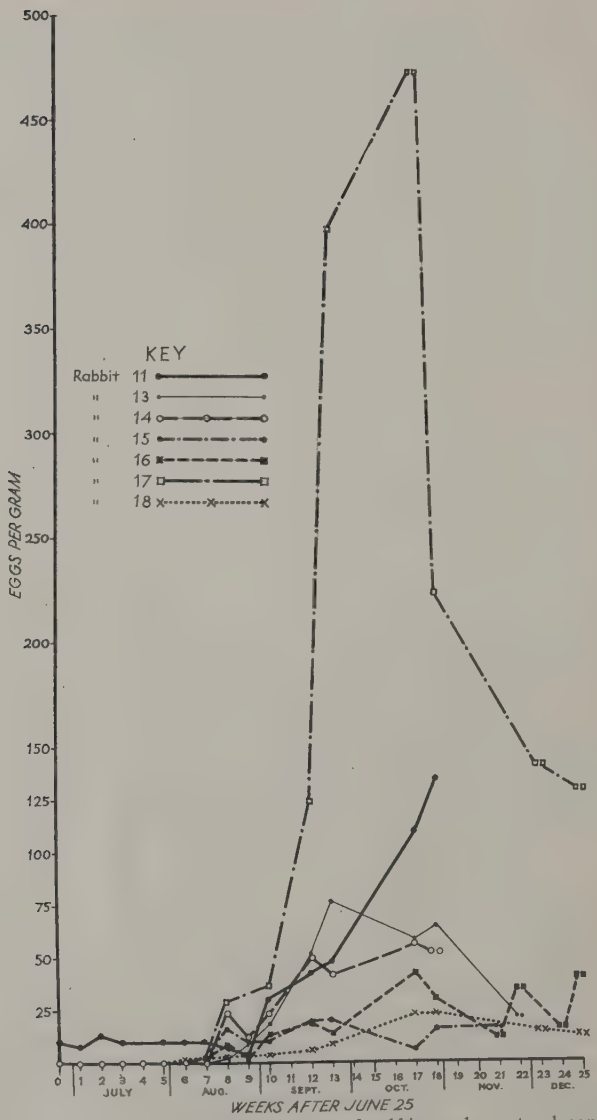
On June 25, Rabbit 11, then about 4 months old, weight 1500 grams, and showing a count of about 10 *T. calcaratus* eggs per gram of feces, was placed in the area, together with 6 clean young rabbits, numbers 13 to 18, litter mates, about two months old. These young rabbits averaged 1215 grams weight each (range 1050 to 1370). The experi-

ment continued until November 15th, by which time temperature conditions were unfavorable for the free-living stages of the worms. An attempt was then made to continue reinfection areas in boxes of soil indoors, but this was not successful, and after a few days was discontinued. As sufficient interval did not elapse for new free-living stages of the worms to develop, it is not considered that the animals were subject to re-infection after coming from the area. In the 20 weeks of the experiment the thrifty condition of the animals was attested by an approximate doubling of the weights of the 6 young animals, to an average of 2280 grams.

As noted, twenty-four hour collections were made each week, although no collections were made the 14th, 15th and 16th weeks (October in part), and those of the 11th week became unusable through inadvertent drying in the laboratory. Figure 1 gives the egg count curves on a per gram basis. As occasional eating of feces by animals in the collection cages was observed, it was not considered feasible to utilize the twenty-four hour total output data as representing a more accurate determination than the eggs per gram. It may be mentioned however that the trend of the eggs per day curves as made up from these collection data differ in no essential respects from those of the eggs per gram shown in Figure 1. Fortunately the high count of Rabbit 17 was determined by a forty-eight hour collection, and is thus unquestionable. It is possible that certain of the animals had higher counts in the 14–16 week interval which were missed by omitted collections, although their possible occurrence does not seriously impair the net result of the experiment. On the 18th week Rabbit 11 was withdrawn from the area for another purpose. Rabbit 14 was killed at the same time and had a post-mortem count of 15 adult *T. calcaratus* (2♂ 13♀). Rabbit 15 was killed the 22nd week and showed 8 adult worms, all females, rabbit 16 the 25th week and showed 6 adults (3♂ and 3♀). The remaining animals were reserved for other uses.

From the experimental data as shown graphically in Figure 1, the following observations are of interest:

1. For several weeks the egg count of the infected Rabbit 11 stayed at a constant level, and the other animals remained negative. This accords with expectation, as it constitutes the period during which the area is becoming seeded with infective stages of *T. calcaratus*, and the required interval between the acquirement of worms by the hosts and the time such worms become functional adults. The consecutive "negatives" indicate also that the rabbits did not ingest eggs from the pellets of Rabbit 11. Inferentially, therefore, later egg counts are from established worms and not by contamination.



Text Figure 1. Eggs per gram counts of rabbits under natural conditions of re-infection with *Trichostrongylus calcaratus*, a small gut nematode. Circles on the baseline indicate "negatives," *i.e.*, no eggs in the feces. Double spots represent 2-day collection averages. Rabbits 13–18 were litter mates, 2 months old at the beginning of the experiment.

2. The gradual development of larger egg counts in all the animals indicated a more or less steady rate of re-infection, which was slow for most of the rabbits. To just what degree this was conditioned by the particular temperature and rainfall of the period need not concern us in this experiment, (although weather conditions in general were held to be favorable for worms), inasmuch as all the animals were kept under identical conditions in one group, and the fact of re-infection is demonstrable. Presumably, and almost surely, re-infection obtained through skin penetration, as the animals were fed indoors on the clean cement floor. So far as the writer is aware, this is the first demonstration of re-infection under natural conditions with a nematode parasite of the rabbit.

3. There is clearly a difference, as measured by egg counts, in the susceptibility of the individual animals to worms becoming established. This is demonstrably not a function of age as such, with 6 of the animals litter mates. Nor is differential exposure to infective worms a useful assumption, for in addition to the fact that the hosts were all in a group and handled alike, there were no individual differences in behavior noticed which, independently of the egg count evidence, would have suggested differential exposure in the 100 square feet of soil area.

4. Besides the fact of re-infection taking place, and variations in its rate with different hosts, there is in one animal, Rabbit 17, a clear indication of the development of a resistance resulting in a precipitate loss of worms. In less clear-cut fashion a fall in egg count is demonstrable in Rabbit 13. Hint of it may be present in certain of the others, although presumably re-infection conditions were not sufficiently favorable toward the end of the season to force host response, provided one has a right to expect such a response uniformly. The high count of Rabbit 17 reached 475 eggs per gram, and 17,000 per day.

Inasmuch as this experiment was preliminary to a more detailed study of *T. calcaratus* and its rabbit host by Dr. M. P. Sarles in this laboratory, questions of pathogenicity, egg-worm correlations, etc., need not be gone into here, for they form part of the content of his studies.

CONCLUSION

An experiment is reported briefly in which rabbits, exposed under "natural" conditions on soil to re-infection with *Trichostrongylus calcaratus* were shown to develop infections of increasing size, as judged by egg count. Considerable individual host variability in accepting worms was apparent, and in at least one instance a precipitate drop in egg count indicated the development of resistance against the infection, and discharge of worms.

References Cited

Sarles, Merritt P. 1932.—Development of an acquired resistance in rabbits by repeated infection with an intestinal nematode, *Trichostrongylus calcaratus* Ransom, 1911. Jour. Parasit., 19:61–82.

Stoll, Norman R. 1929.—Studies with the strongyloid nematode, *Haemonchus contortus*. I. Acquired resistance of hosts under natural reinfection conditions out-of-doors. Amer. Jour. Hyg., 10:384–418.

1930.—On methods of counting nematode ova in sheep dung. Parasitology, 22:116–136.

DEVELOPMENT OF AN ACQUIRED RESISTANCE IN RABBITS BY REPEATED INFECTION WITH AN INTESTINAL NEMATODE, *TRICHOSTRONGYLUS CALCARATUS* RANSOM, 1911.*

MERRITT P. SARLES

In a short paper dealing with reinfection of the domestic rabbit with a gut nematode, *Trichostrongylus calcaratus,* under natural conditions, Stoll (1932) presented evidence that in some cases at least this host illustrated an effect he had demonstrated earlier (1929) with domestic sheep and a stomach worm, *Haemonchus contortus;* namely, an initial development of parasitism followed by an apparent host response with discharge of worms. The present article deals with a further exploration of this reaction in a small group of rabbits kept under laboratory conditions precluding natural reinfection, but given definite increasing doses of *T. calcaratus* larvae at regular intervals, and followed by frequent counts of the number of parasite eggs in the feces in order to determine the ultimate effect on both hosts and parasites.

Investigation of acquired immunities to parasitic nematodes is comparatively recent. Sandground (1928) found an immunity in dogs and cats to a second infection of *Strongyloides stercoralis,* an intestinal parasite of man, but regarded it as probably due to the fact that the parasite was in an abnormal host. Stoll (1929) emphasized the importance of studying animals exposed to repeated infection since "the usual hosts of nematode parasites are not infected just once in their lives, but receive more or less constant increments of additional parasites, at least seasonally." He demonstrated that sheep exposed to such reinfection with *H. contortus* developed a resistance, or self-cure and protection, and stated that this phenomenon, if of general occurrence among other hosts and parasites, would necessitate a revision of the generally accepted ideas concerning helminthic infections. The advance abstract of the present paper (Sarles, 1930) indicated that the phenomenon was not limited to sheep, and predicted that it could accordingly be expected to occur with other hosts and worms when appropriately studied. This statement has been substantiated by subsequent reports of acquired resistance to parasitic nematodes, many of which may be interpreted on the basis of self-cure and protection.

McCoy (1931a) studying *Ancylostoma caninum* in dogs and cats demonstrated what he interpreted as an "immunity reaction" following repeated infection. Casual evidence of increased resistance in dogs

* From the Department of Animal and Plant Pathology of The Rockefeller Institute for Medical Research, Princeton, New Jersey.

after previous infection with hookworms had been reported earlier by Payne (1923), Herrick (1928), and Sarles (1929), using *A. caninum,* and by Fülleborn (1926), using *Uncinaria stenocephala.*

An acquired resistance in rats following infection with *Nippostrongylus muris* has been reported by Schwartz, Alicata and Lucker (1931), Africa (1931), and Chandler (1932). Rats also acquire a relative immunity following infection with *Trichinella spiralis,* as shown by McCoy (1931b). An acquired resistance in chickens to infection with *Ascaridia lineata* has been reported by Graham, Ackert, and Jones (1932).

MATERIALS AND METHODS

The parasite used in this study, *Trichostrongylus calcaratus* Ransom (1911) is one of the bursate nematodes with the following life history: entrance into the host as a filariform larva; growth to adult stage in a relatively localized area of the gastro-intestinal tract (in this case principally the jejunum); mating of males and females and production of large numbers of eggs into the lumen of the gut from whence they escape into the feces; embryonation and hatching of the eggs in the dung, and development into the infective larval stage after a week or two under favorable moisture and temperature conditions. Stoll (1932) has shown that infections of *T. calcaratus* may result from the larva penetrating through the skin, or by ingestion.

The fact should be emphasized that there is no multiplication of the worms within the host. One egg develops into one larva, which after developing to the infective stage may parasitize a host and become one adult male or female worm. Hosts which harbor large numbers of worms have, therefore, necessarily received large numbers of larvae. Under natural conditions the number of eggs shed by the individual female worms gradually permit an extensive number of infective larvae to develop and reinfect the host. The numerous eggs given off into the gut by the adult female worms permit the experimenter to follow the degree and fate of the infestations by the simple means of egg counting the feces.

Inasmuch as rabbits from our laboratory stock occasionally show light infestations of *T. calcaratus* and *Obeliscoides cuniculi* (a stomach worm), we obtain them when they are weaned at 6 weeks of age, and only use for experimentation those which show no worm eggs in their feces during a subsequent period of at least 3 weeks. Such animals can be reasonably assumed to have never been infected with the two above species. The 7 rabbits reported in this paper showed only coccidial oocysts upon weekly examination of their feces by flotation during the 3 to 6 week period which elapsed from the time that they were weaned until their first infection. They were kept in metal cages

throughout the experiment, on one-half inch mesh wire screens fastened over the top of a metal pan. The screens were scrubbed daily, and the rabbits were transferred each week to a clean set of cages which had been sterilized in boiling water. In one rabbit (No. 110) small numbers of the eggs of the rabbit oxyurid, *Passalurus ambiguus,* were noted at two different periods during the experiment. It does not seem probable that the oxyurid or coccidial infection had any appreciable influence on the outcome of the experiment. A control rabbit remained uninfected throughout the experiment indicating that no infections were being picked up from food or in cage transfers.

Screens supporting the rabbits allowed the fecal pellets to fall on moistened paper towels in the 3 inch deep pan below, from which they were collected daily and their consistency noted. The 24 hour specimen was weighed, a portion taken for cultures and the remainder either used immediately for making up egg count flasks or kept under refrigeration until used. Two egg count flasks were made up using 10 grams of pellets to 300 cc. of diluent: a water flask for flotation counts and a sodium hydroxide flask for dilution egg counts. The manipulation and counting of these flasks were in strict accordance with the steps recently outlined and discussed in detail by Stoll (1930). All counts were made by the author, the collection and tubing of specimens being done by an experienced technician under supervision. The one pipette used for all the dilution egg counts, together with all flasks, etc., were calibrated against Bureau of Standards certified pipettes. The flotation method was used to detect the first appearance of eggs in the feces and for counts under 200 eggs per gram, whereas the dilution counting technic was utilized for all larger counts. The flotation counts were carefully established and checked by counting 3 covers from each of 2 tubes, i. e. the eggs from one gram of feces were enumerated. The dilution egg counts may also be regarded as being highly reliable since they were based on the counting of 2 to 9 large drop (0.15 cc.) slides from each flask, with an average of 4.5 slides, utilizing sufficient slides to reach the 5 per cent limit (Stoll, 1930) on 78 of the total series of 91 flasks and the 10 per cent limit on 11 other flasks. It is not feasible to present in this paper the results of a statistical analysis of the egg count data secured beyond the statement that both of the counting methods employed were demonstrably satisfactory for enumerating *T. calcaratus* ova in rabbit dung.

Originally it had been planned to make daily counts on all of the rabbits but this was soon found impracticable, and the number was reduced to 2 or 3 a week, except in the case of Rabbit 103, which was followed daily from the 14th to the 139th day. In the final weeks of the experiment counts were not made oftener than once a week.

The larvae used for the first 2 infections were cultured from one of the animals earlier reported by Stoll (1932). Larvae for later infections were cultured from the feces of the 6 rabbits of the present experiment, and from 4 other originally "clean" rabbits infected from them. High and uniform infectivity of the larvae, and purity of the parasitic species throughout the study were proven by the nature of the infestations produced in 12 other rabbits infected with larvae from the same cultures used in this experiment, and by the recovery of only *T. calcaratus* from these animals at autopsy.

To secure infective larvae, fecal pellets from infected rabbits were left in a Petri dish, moist chamber, or pasteboard carton, at room temperature for 10 days and then isolated in the Baermann apparatus (Cort, et al, 1922). The larvae were then washed several times in tap water and counted under the microscope, using a 40 mm. objective and a 10× ocular. Doses up to 400 larvae were counted direct, but larger doses were done by dilution, by counting two or more one-half cc. samples from a larval suspension of 10, 25, or 50 cc. The degree of accuracy of these dilution counts was ascertained by the method of Scott (1928) for each of the 3 dilutions that were used. For suspensions of 10 cc., which were used to count doses of 400 to 4,000 larvae, the coefficient of variation of the average of two counts was 12.5 ± 1.2 per cent; on suspensions of 25 cc. and 50 cc., which were used to count doses of 1,000 to 21,000 larvae, the coefficient of variation was 8.5 ± 0.8 per cent.

Mouth infections were given by holding the rabbit's jaws open and pipetting into the back of the mouth a few drops of water containing the larvae. The rabbit swallowed readily in most instances, with only occasional slight loss of fluid. The skin infections were made by clipping the hair as closely as possible over an area 3 to 4 cm. in diameter, placing the larvae on this area in a thin film of water and allowing the water to evaporate slowly (15 to 30 minutes), while the animal was held in one position. Each successive cutaneous infection was made on a previously unused area of the body.

The ages of the rabbits when first infected November 16, 1929, were three months for Rabbits 103 and 104, which were litter mates of the control, and two and one-half months for Rabbits 110, 111, 112 and 113, also litter mates. The second series of infections were given when the animals were 21 weeks older.

The rabbits were kept on a uniform diet consisting of a daily ration of one-half pint grain mixture, one-fourth pint moistened beet pulp, and 4 to 8 ounces of green cabbage, fed in door cups each morning, with water given freely. On this diet, which appeared to be adequate, the rabbits produced formed fecal pellets that crushed easily in the egg

count flasks and contained very little coarse vegetable débris. Such dung has been found optimum for convenience and accuracy of egg counting and well suited for the culturing of *T. calcaratus* larvae.

It was planned to build up infestations in the 6 rabbits by giving weekly doses of increasing numbers of larvae; 3 animals to be infected by mouth, and 3 by skin. Partly for convenience, and partly to simulate the assault of an increasing number of parasites, such as hosts might be exposed to when on limited areas out-of-doors, each succeeding dose was to be the sum of all earlier ones. As a matter of fact, the requirements for infective larvae outstripped our ability to culture them at first, when we were dependent upon a single animal for cultures, and also towards the end of the initial series of infections when the practically simultaneous loss of worms by 5 of the 6 rabbits was reflected in a rapidly decreasing yield of larvae from cultures. Consequently it was impossible to maintain the weekly doses during the first two months of the experiment, and some of the final doses were not increased in size to the degree planned. With these exceptions the plan of weekly additive doses was closely followed, as may be seen from Figures 1–3 and 6–8. This method of infection is a process by which, substantially, the experimenter assists in developing, with a parasite which does not naturally multiply in its host, total infestations of a size comparable to what might have obtained by a parasite multiplying *in vivo*. As it was known that this parasite has the potentiality of being fatal for its host when present in large numbers, such a building up of the worm burden provided a method of forcing a host response.

RESULTS

The course of the infestation produced in each of the 6 rabbits by the doses of larvae which it received are shown in Figures 1–3 and 6–8, which are graphs of the eggs per day counts listed in Tables 1 and 2. (The infections showed the same course in terms of eggs per gram counts as used by Stoll (1932), but the counts were somewhat more variable from day to day.) In these figures the crosses indicate the number of eggs which were passed on each day that a count was made (except in the case of Rabbit 103 where they represent 3-day moving averages of daily counts) while the open circles on the base line show that an examination was negative. At the top of the graphs are listed the number of larvae given at each successive dose. The arrows indicate the size of these doses and when given, and also constitute a theoretical curve of expected egg production based on the number of eggs which appeared following the first dose of larvae. The first dose given to the 3 orally infected rabbits produced average egg counts for the 3rd week after infection of 23, 32 and 28 eggs per day per larvae, with

TABLE I.—*Eggs Per Day Counts (in Thousands)*

Days after first inf.	Rabbits					Days after first inf.	Rabbits					Days after first inf.	Rabbits			
	104	110	111	112	113		104	110	111	112	113		110	111	112	113
3	N	N	N	N	N	44	0.3	3	0.2	1	1	111	–	–	51	N
10	N	N	N	N	N	47	–	4	–	2	–	114	N	0.1	86	N
13	N	0.1	N	N	N	48	–	3	–	3	–	121	1	2	15	N
14	N	0.1	N	N	N	49	–	–	–	5	–	128	8	N	7	N
15	N	0.2	N	0.2	N	50	–	–	–	3	–	135	N	N	11	0.7
16	0.3	0.7	0.1	0.5	N	51	0.3	3	0.5	8	0.4	142	N	N	10	4
17	0.1	1	0.1	1	N	54	0.5	2	0.4	5	0.5	149	N	N	22	4
18	0.2	1	0.3	1	N	58	0.6	4	0.3	4	0.2	156	N	N	9	1
19	0.7	1	0.3	4	N	61	0.2	3	0.3	10	0.3	158	N	N	13	2
20	0.5	2	0.1	2	N	65	0.4	10	0.5	15	0.4	160	N	N	17	0.5
21	0.1	4	0.1	1	N	67	0.5	10	0.9	13	1	163	N	N	7	0.2
22	0.2	2	0.1	0.4	N	69	0.8	13	0.4	20	1	165	N	N	14	N
23	0.3	4	0.6	4	N	72	0.6	23	1	62	2	167	N	N	14	N
24	0.5	3	3	2	N	74	0.5	21	3	81	1	170–1	N	N	7	N
25	0.1	–	0.5	2	N	76	0.4	21	1	50	2	177–8	N	N	16	7
26	0.5	3	0.4	2	0.1	79	2	45	1	69	1	191–2	N	N	17	N
27	0.1	3	1	1	1	81	1	16	1	74	2	212–3	N	0.8	18	7
30	0.4	4	0.4	2	0.1	83	3	22	1	79	7	219	0.1	0.1	–	1
31	0.2	2	0.5	2	0.5	86	7	12	2	5	7	226–7			15	
33	0.1	2	0.6	2	1	88	–	17	–	N	–	254–5			16	
34	0.2	1	0.3	1	0.5	90	9	0.1	2	0.2	10	282			N	
36	0.6	2	0.4	3	0.1	93	24	N	2	0.2	15					
37	0.5	3	1	1	0.7	97	24	–	4	–	18					
40	0.4	5	1	3	0.7	100	10	N	5	1	34					
41	0.4	1	1	2	1	104	2	–	0.1	–	31					
43	0.3	1	1	1	0.4	107	N	N	N	11	15					

N = Negative for eggs.

a mean of 28, which was the factor used for plotting the dosage arrows for all oral infections. The first dose given the 3 cutaneously infected rabbits yielded for the same period 6, 4, and 0 eggs per day, with a mean of 3.3, which was the factor used for plotting the dosage arrows for all skin infections. As it takes approximately 2 weeks for the larvae to develop into adult worms and produce eggs, and another week for the count to reach its maximum, each arrow represents the increase in the egg count which might be expected during the 3rd week after that infection if the rabbit were as susceptible to all doses as it was to the first one. The factor used for the oral infections is a very conserva-

TABLE 2.—*Daily Egg Counts on Rabbit 103 (in Thousands)*

Days	EPD	Days	EPD	Days	EPD	Days	EPD	Days	EPD
3	N	40	4	68	62	96	63	124	87
10	N	41	1	69	58	97	66	125	90
11–13	N	42	2	70	62	98	34	126	65
14	N	43	3	71	82	99	75	127	100
15	1	44	2	72	132	100	51	128	100
16	1	45	1	73	114	101	92	129	74
17	0.1	46	2	74	109	102	41	130	103
18	1	47	3	75	96	103	21	131	136
19	1	48	1	76	65	104	27	132	69
20	1	49	3	77	96	105	17	133	72
21	1	50	2	78	93	106	44	134	79
22	0.04	51	1	79	94	107	17	135	70
23	3	52	3	80	162	108	53	136	69
24	1	53	3	81	103	109	73	137	226
25	0.1	54	4	82	189	110	66	138	52
26	1	55	7	83	139	111	117	139	43
27	1	56	3	84	101	112	40	142	73
28	1	57	3	85	105	113	45	144	9
29	1	58	7	86	101	114	70	146	0
30	0.4	59	7	87	84	115	66	149	0
31	0.3	60	4	88	75	116	59	151	0
32	1	61	8	89	72	117	25	153	0
33	1	62	11	90	77	118	37	156–7	0
34	1	63	8	91	90	119	35	163–4	0
35	1	64	28	92	101	120	36	170–1	0
36	2	65	26	93	112	121	73	177–8	0
37	1	66	107	94	93	122	87	191–2	0
38–39	1	67	63	95	41	123	111	212–3	0
								219	0

EPD = eggs per day. N = negative for eggs.

tive one when compared with that of 43 eggs per day per larva produced by the single infection of 2,500 larvae given to Rabbit 116. Accordingly the failure of the egg counts to increase with the theoretical curve is somewhat minimized.

Rabbit 103. Figure 1 shows the egg counts (as moving 3-day averages of daily counts) which resulted from a series of 18 oral infections. The changes in the total number of eggs passed per day are seen to occur in weekly periods or cycles starting on or a day or two before the 14th day after each infection (which is the period required for the larvae of *T. calcaratus* to develop into adult worms and produce eggs). The first 8 doses of larvae produced rises in the egg count

roughly proportional to the number of larvae given, but then, despite the giving of larger and larger doses, the count fell off precipitously in the 13th to 15th week. A second rise in the count occurred in the 16th week (evidently induced by the large 12th dose of 26,000 larvae) but was followed by a drop in the 17th week. The egg count mounted a third time in the 18th and 19th weeks, in evident response to the

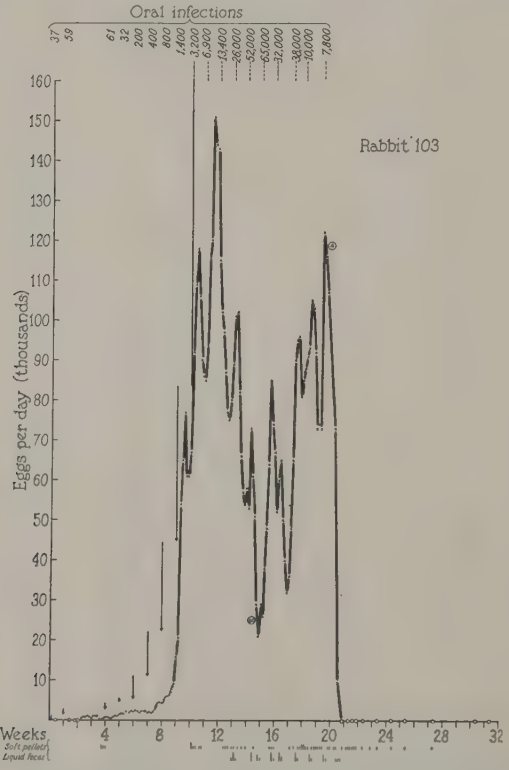


Fig. 1. Infestation record of Rabbit 103, showing initial susceptibility to infection, incomplete elimination of the infection followed by breaking through of the large doses of larvae, and finally complete elimination of the infection. Numbers in circles indicate adult worms recovered from feces. At bottom of chart are shown the association of passage of soft pellets with infection, and liquid feces with decrease in the egg count.

large 14th and 15th infections of 65,000 and 32,000 larvae, but dropped sharply to a negative in spite of two additional infections, and was consistently negative on 10 examinations during the following 11 weeks.

The reduced scale graph of this same infection (Figure 4) shows the contrast between the actual curve of egg production and the curve which would have been expected had the rabbit remained as susceptible to all the doses of larvae as it was to the first one. We interpret these egg counts as indicating that the rabbit was initially susceptible but that repeated infections developed a resistance which threw off the greater part of the worms. Some of the larger doses temporarily broke through this developing resistance. However, the rabbit not only failed to show a proportional increase in amount of infection when assaulted repeatedly with very large numbers of infective larvae, but finally completely rid itself of the infection.

The failure of the huge doses of larvae to produce comparable rises in Rabbit 103's egg count may be partly explained by the fact that a large proportion of the larvae passed directly through the animal during the two days following these infections. Thus 62 per cent of the 13th dose of larvae was recovered, as counted by dilution, 30 per cent of 14th dose, 18 per cent of the 15th dose, and 21 per cent of the 16th dose. (Passage of infective larvae through the alimentary tract has been previously observed by Stoll (1929) when large doses of *Haemonchus contortus* larvae were fed to sheep.)

Rabbit 110. Figure 2 shows that the number of eggs passed by

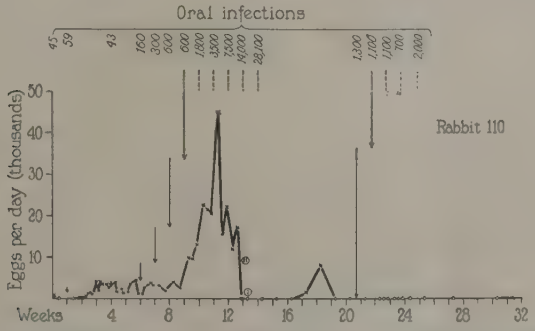


Fig. 2. Infestation record of Rabbit 110, showing initial susceptibility, occurrence of crisis and self-cure, and resistance to a second series of infections.

this rabbit increased with the number of larvae given for the first seven infections but then dropped off to a negative despite the larger and larger doses. Evidently a few worms from some of the last of these oral doses temporarily broke through the resistance which the animal had developed and produced a small egg count in the 18th and 19th weeks. A second series of 5 small oral doses, given to test the animal's resistance, produced only a delayed and barely detectable count. The chart of this infection at a reduced scale (see Figure 4) shows

the enormous disproportion between the amount of infection which the animal should have taken had it been uniformly susceptible and the amount which it actually acquired and then eliminated.

Rabbit 112. In this orally infected animal (Figure 3) only the

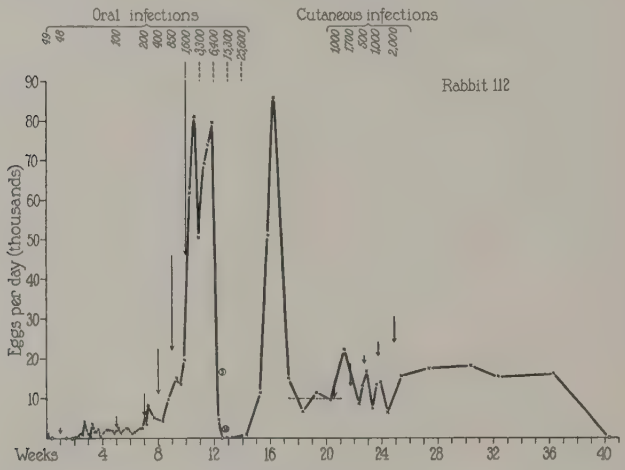


Fig. 3. Infestation record of Rabbit 112, showing initial susceptibility, self-cure, breaking through of heavy doses leaving a residual infection, relative immunity to a test series of skin infections, and ultimate elimination of the infection.

first six doses of larvae added increments to the egg count which followed the larval curve, the count dropping to a negative in the 13th week after infection. A small count reappeared, however, and evidently the large 10th and 11th doses both broke through the rabbit's newly developed resistance to build up a high count in the 16th to 17th week. This secondary peak of infection was short lived but it left a residual infection (shown in Figure 3 by the dotted line at the 18th to 21st weeks) of about 10,000 eggs per day, upon which were superimposed 5 weekly doses *by skin* of between 500–2,000 larvae each. Apparently the count decreased during the time of these infections instead of increasing, showing that the animal was resistant to superimposed infection. At the termination of these test doses the infection established itself at a slightly higher level than before and remained there for some time. When the animal was examined a final time it had become negative, and no worms were found in the intestinal tract at autopsy six days later. The chart plotted at a reduced scale (see Figure 4) to show the complete theoretical curve of expected egg production

illustrates the failure of the host to become parasitized by more than a very small fraction of the larvae which it received.

The gradual increase in the number of eggs passed in the dung of the 3 rabbits given an initial series of increasing infections by mouth, and the later abrupt drop in the egg count may be contrasted with the egg counts of Rabbit 116 (see Figure 5). This animal received a single

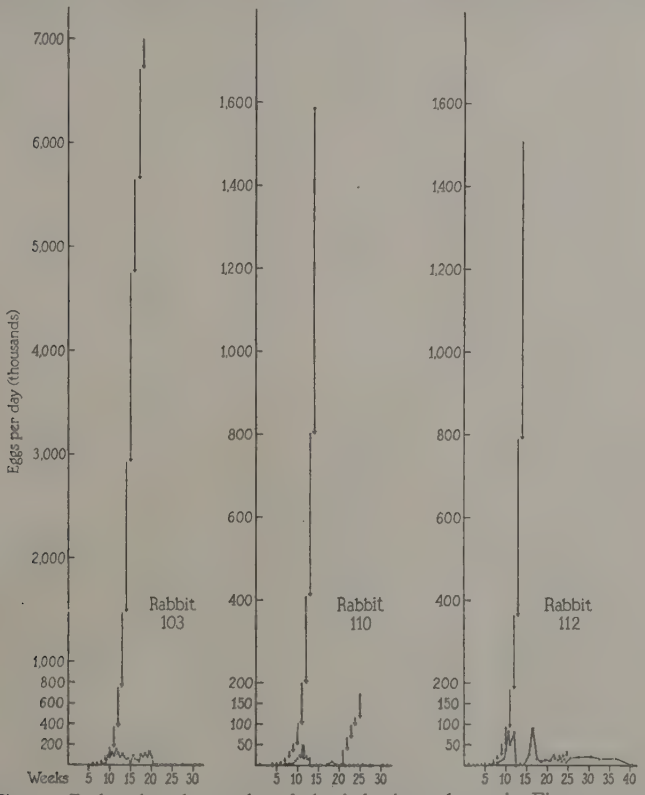


Fig. 4. Reduced scale graphs of the infections shown in Figures 1–3 contrasting the small size of the actual egg counts with the theoretical counts, indicated by the arrows, which would have resulted if the rabbits had remained as susceptible to all doses of larvae as they were to the first dose.

dose of 2,500 *T. calcaratus* larvae by mouth at the time when a dose of approximately the same size and of the same larvae was given to Rabbits 103, 110 and 112, as one of a series of doses, at 10 weeks after their first infection. In Rabbit 116, which had been found repeatedly negative for worm eggs from the time it was weaned until infected at four and one-half months of age, eggs appeared first on the 13th day

and the count mounted very rapidly during the 3rd and 4th weeks to a peak of 108,000 eggs per day, and then declined gradually over a period of nearly a year, becoming permanently negative 49 weeks after infection. (When the decreasing egg counts of this animal were plotted on arith-logarithmic paper they approximated a straight line, from which the smooth curve $Y = 156{,}000\ (.86)^x$ of Figure 5 was fitted graphically, indicating that the infection was being lost at a constant rate.) This sharp rise in the egg count, and the following gradual decline are typical for single infections of this size. In the other 3 rabbits

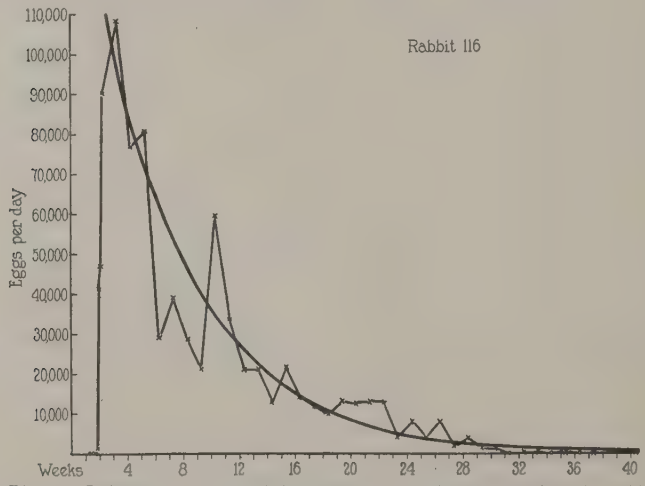


Fig. 5. Infestation produced by giving a previously uninfected rabbit a single oral infection of 2500 *T. calcaratus* larvae. Contrast the constant rate of loss of this infestation with the abrupt elimination of infestation in the 6 repeatedly infected rabbits.

which received roughly the same doses, as only one of the smaller doses in their initial series of infections, there was no increase in count that could be attributed to this infection, and, on the contrary, the change in egg count which occurred in the 13th week, when eggs from this infection should have been expected to appear, was in every case a decrease, as may be seen in Figures 1, 2 and 3. The *whole series* of doses fed to Rabbits 103, 110 and 112 produced only in Rabbit 103 a count as high as that occasioned by the single dose given to Rabbit 116.

Rabbit 104. This animal received 11 infections by skin (Figure 6), but due to a shortage of larvae it and the other two infected by skin received no doses of larvae for 5 weeks after the first two doses. There was very little increase in the small count arising from the first two infections until the sixth infection was given. This dose and the next

one were followed by proportional increases in the egg count, which then dropped sharply to a negative in spite of three final infections of large numbers of larvae. The animal was autopsied the day after it became negative, and only 17 adult *T. calcaratus* were found although the rabbit had received a total of 38,000 larvae. If the animal had remained uniformly susceptible throughout the experiment the egg count would have reached the height shown by the arrows.

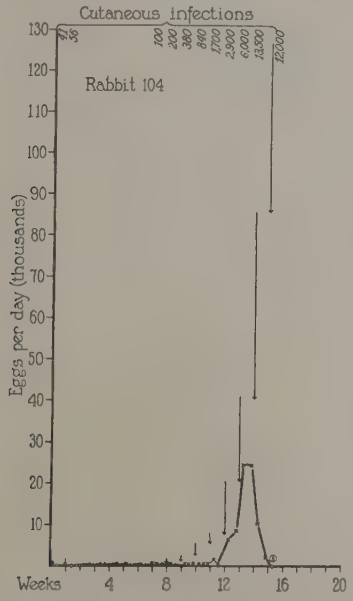


Fig. 6. Infestation record of Rabbit 104, showing occurrence of self-cure following repeated cutaneous infection.

Rabbit 111. The number of worms colonizing the intestines of this animal, as indicated by the egg count (Figure 7), increased with the number of larvae given at first, then lagged behind, and finally dropped to a negative in the 16th week. The two final doses of the first series were evidently responsible for the slight temporary increase in the count during the 17th and 18th weeks. Five weekly doses of larvae given *orally* as a crucial test of this animal's resistance produced only a delayed and barely detectable egg count, whereas in a previously uninfected animal the count shown by the arrows would have been expected. This rabbit was the least susceptible to repeated infection of any of the 6 animals and demonstrated conclusively that a resistance acquired against skin infection also provides effective protection against mouth infection.

Rabbit 113. The first skin infection given this animal apparently did not induce any detectable infection, but the second to eighth doses inclusive produced proportional egg count increases (Figure 8). In contrast to this, the final three doses were followed by a rapid decrease in the count to a negative. Some of the larvae of these last three infections were probably responsible for the small and delayed count which appeared in the 20th, 21st and 22nd weeks. The resistance which had thus manifested itself was tested by a second series of 5 small weekly doses of larvae also given by skin. The egg count decreased while

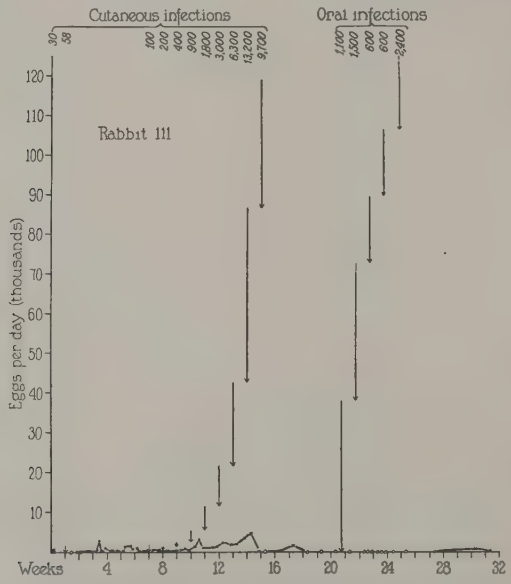


Fig. 7. Infestation record of Rabbit 111, showing early development of resistance to cutaneous infection and resulting refractoriness to oral infection.

these were being given. The two transient increases which occurred following this second series of "test" doses may be interpreted as the breaking through of an infection followed by reestablishment of the animal's resistance.

Worms recovered from feces. Five of the 6 repeatedly infected rabbits passed adult worms in their dung at the time when the sharp drop occurred in their egg counts, proving that the decrease in the count was actually associated with loss of parasites. The worms were recovered by screening the crushed feces through screens of 20, 40 and 60 meshes to the inch. The total number of worms calculated to

have been passed by each rabbit on each day that worms were recovered is shown by the numbers in circles in Figures 1 to 3 and Figures 6 to 8. These worms, recovered by the occasional screening of a comparatively small portion of the total feces passed by the rabbits, obviously represent only part of the total number of parasites which must have been eliminated to account for the great decreases in egg counts which occurred at the time the worms were found. That this passage of worms is not merely evidence of a normal turnover in the parasitic population of an animal exposed to repeated infection is shown by failure to find worms immediately before and after these sudden drops

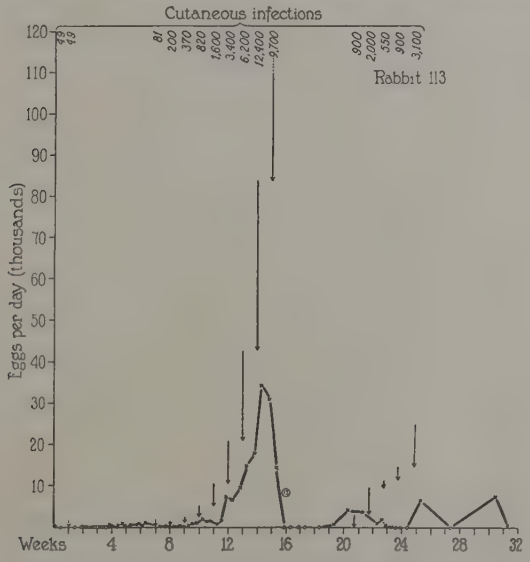


Fig. 8. Infestation record of Rabbit 113 illustrating the acquirement of resistance to superinfection, occurrence of self-cure, and relative resistance to a second series of infections.

in the egg count, and by the more detailed data concerning Rabbit 103. At least 10 per cent of the dung passed each day by this rabbit from the 88th through the 101st day after its first infection was screened for worms but none were found. For this interval the egg count averaged 74,000 eggs per day. But when the egg count dropped on the 104th day to 26,500 eggs per day on a 102 gram specimen, of which 31 grams were liquid, 6 worms were recovered from 10 grams of the mixture, giving an estimated number of 60 worms in the total specimen.

Passage of soft pellets and liquid feces. Parasitic infection and the development of self-cure and resistance in the rabbits were asso-

ciated with the passage of soft pellets and liquid feces. This is best shown by daily information secured on Rabbit 103. At the bottom of Figure 1 are indicated the days on which feces of either of these two consistencies were found in addition to the usual formed pellets. The short bars indicate that a small amount of the day's fecal specimen was of the designated form, the large ones that a relatively large proportion was passed. Before infection, and throughout the first 10 weeks after infection, the animal passed firm fecal pellets almost exclusively, but from the 10th week on masses of soft pellets occurred very frequently and diarrheic feces were observed every week from the 14th through the 21st. The worms which were recovered by screening Rabbit 103's feces were all from liquid specimens. There is not as detailed a record for the other five rabbits, but they, too, show a correlation of soft pellets and diarrheic feces with infection and loss of worms. This change in fecal consistency was not caused by diet, since it did not occur in the control rabbit, nor can it be laid to coccidial infection, as the animals had ceased a pass oocysts. Stoll (1929) observed a similar passage of soft pellets by sheep at the height of *Haemonchus contortus* infections.

Skin lesions. The only other clinical sign of the rabbits' reaction to repeated infection (their weight curves were not significantly different from that of the uninfected control) was the appearance of small nodules at the site of infection following some of the larger doses given by skin. Rabbit 104 and Rabbit 111 developed nodules after the 7th, 9th, 10th, and 11th skin infection, and Rabbit 113 showed them after the 9th and 10th doses of the first series, and after the 3rd and 4th test doses. They appeared within a few days after infection as papules of 2 to 5 mm. in diameter. They were firm, movable, yellow in the center, circumscribed with a narrow red zone, and blanched on pressure. They decreased in size for 2 or 3 weeks, becoming dry and white, and finally sloughed away leaving a pigmented spot in the skin.

DISCUSSION

Although the most striking characteristic of the infections produced in the 6 rabbits given increasing weekly doses of larvae may seem at first to be their dissimilarity, careful study of Figures 1 to 3 and Figures 6 to 8 will show that the general course of the infection was the same in each animal and that the differences were merely a matter of degree. All 6 were initially susceptible as shown by the establishment of an infection and its increase with the increase in the doses of larvae. All became resistant to superinfection after 6 to 8 doses of larvae, and this phase of their immunity development was uniformly followed by an abrupt drop in their egg counts associated with the passage of

worms in their feces. In each animal this process of self-cure was followed by the temporary establishment of a new infestation resulting from the thousands of larvae which were being given at the culmination of the first series of doses. These secondary peaks of infection were in turn more or less completely reduced and the resistance reestablished as shown by the rabbits' relative resistance to a second series of doses. The increase of the egg count to a maximum occurred at the same time in all of the animals infected by the same method, although later in those infected by skin (first peak of infection at the 14–15th week) then in those infected by mouth (first peak of infection at the 11–12th week) because of the five week period when no larvae were given to the former group. The first sudden decrease in the egg count curves, indicating the occurrence of self-cure, also took place at roughly the same time in all rabbits of each group, occurring in the 12–15th week in the orally infected animals and in the 15–16th week in the cutaneously infected ones.

Obvious differences between the infections of the 6 repeatedly infected rabbits are shown in the general shape of the egg count graphs, and in the size of the egg counts. Since the number of larvae received by each rabbit was not the same the latter are better compared in terms of the egg-larva ratio, i. e. by a ratio obtained by dividing the egg count produced by a particular dose or group of doses by the number of larvae which had been given a sufficient time previously (at least two weeks) to have played a possible part. Table 3 gives such egg-larva ratios for each animal and also summarizes other comparative data. It will be seen from this table that infections given by skin took longer to appear than those given by mouth. The egg-larva ratio calculated for the total ascending portion of the egg count curves, or period of initial susceptibility, was 41 for oral infections and only 4.4 for cutaneous infections. The egg-larva ratio for Rabbit 116, given a single dose of 2,500 larvae, was 43. Thus during the early phase of the infection repeated doses of larvae were equally as effective in establishing worms in the three orally infected rabbits as a large dose of larvae given all at once to another animal.

The egg-larva ratios for individual animals during this same period show that Rabbit 110 was less susceptible to infection than the other two orally infected rabbits and Rabbit 111 less susceptible than the other two infected cutaneously. It is of interest that the later behavior of the rabbits is in accordance with their comparative susceptibility classification. Thus Rabbits 103 and 112 were more susceptible, on the basis of these ratios, than Rabbit 110, and in both the heaviest doses of the initial series of infections broke through their partially established resistance and succeeded in producing large infestations

which were only thrown off after some time, whereas in Rabbit 110, the heavy doses of larvae succeeded only in reestablishing a small infection which was rapidly and completely eliminated. Likewise, among the skin infections, Rabbit 111, which had the lowest ratio and appeared to become resistant very early, showed a slightly and more transient breaking through of the largest doses of larvae than the more susceptible (by ratio) Rabbit 113, and also proved less susceptible to the test infections.

The egg-larva ratio may also be used to contrast the large total number of larvae which the rabbits received in their initial series of doses with the small amount of infection which was left at the time of the test doses. As Table 3 shows, 4 of the 6 rabbits had a zero ratio at this time, being negative for worm eggs, and the other two had ratio of below 0.2 eggs per day per larvae. The most striking case is that of Rabbit 103. This animal had been highly susceptible initially as shown by a ratio of 50 eggs per day per larva for the first 8 infections, totalling 3,000 larvae, but its ratio became zero in the 3rd week after its last infection of 7,800 larvae, although it had received a total of 257,000 larvae.

The purpose of the second series of doses which were given to 4 of the rabbits was to test the resistance to infection which the animals were believed to have acquired as a result of exposure to their initial series of infections and also in 2 rabbits to test for immunity against infection by the alternate method from that previously used. The degree to which the rabbits were susceptible to this second series of doses is shown in Table 3, in terms of egg-larvae ratios based on the maximum increase which was observed in the egg counts. The five test doses given orally to Rabbit 110, which had become negative following a previous series of oral doses, gave a ratio of only 0.02. A similar series of oral doses given to Rabbit 111, which was negative as the result of an initial series of cutaneous doses, gave an egg-larva ratio of only 0.1. These two factors show that both these animals were relatively resistant to infection, since they represent the establishment of an insignificant egg count when compared with the average ratio of 41 for animals susceptible to oral infection. In Rabbit 113, which had eliminated most of its original infestation produced by cutaneous infection, the egg-larva ratio for the maximum increase in egg count produced by the test doses given cutaneously was 0.4. For Rabbit 112, carrying a residual infestation of uncertain level from an initial series of oral doses, the egg count decreased during the time that the test doses were being given by skin, but increased later to a new plateau of infection, which, if brought about solely by the test doses, would give a ratio of 1.3. As the ratio for animals susceptible to infection by skin

TABLE 3.—*Comparative Data Concerning Infestations of Rabbits Repeatedly Infected with Trichostrongylus calcaratus*

	Initial Series of Infections								Test Infections			
Rabbit No.	Infected	1st Eggs (Days)	1st Peak (EPD)	No. doses involved	Total larvae given	Egg-larva ratio	Residual count (EPD)	Total larvae given	Egg-larva ratio	Infected	Total larvae given	Max. increase (EPD)	Egg-larva ratio
103	by mouth	15	151,000	8	3,000	50	0	257,000	0	.....	.....	....	...
110	" "	13	45,000	7	1,800	25	0	57,000	0	by mouth	6,200	100	0.02
112	" "	15	81,000	6	1,650	49	10,000	54,000	0.2	by skin	6,250	8,000	1.3
Average for mouth infections		14.3	94,000			41							
104	by skin	16	24,000	7	3,300	7.3	0	38,000	0	.....	.....	....	...
111	" "	16	5,000	8	6,500	0.8	0	36,000	0	by mouth	6,200	800	0.1
113	" "	26	34,000	8	6,600	5.1	4,000	35,000	0.1	by skin	7,450	3,000	0.4
Average for skin infections		19.3	21,000			4.4							

EPD = Eggs per day.

was 4.4, both rabbits were relatively resistant by this test, as the increase brought about by the test doses was very slight in the case of Rabbit 113, and small, even if solely due to the test doses, in Rabbit 112. Of course any ratio above zero resulting from the test doses, although small in size when compared with the ratio for susceptible animals, is significant in that it demonstrates that the resistance of the rabbits was relative, not absolute. The resistance displayed by Rabbits 111 and 112 to infection by the alternative method from that to which they had previously become resistant is most marked in the case of Rabbit 111, but sufficiently definite in both animals to demonstrate that the protection is not limited to infection by the route used to produce the resistance.

The increasing age of the rabbits during the experiment is not considered to have played any significant part in their development of a resistance to infection since we have failed so far to demonstrate the presence of any marked age resistance in rabbits to infection with *T. calcaratus*.

The occurrence of self-cure and protection in rabbits exposed to repeated infection with *T. calcaratus*, as reported in the present paper has also been observed in a second series of rabbits given larger increasing doses of larvae, as well as in several groups of rabbits exposed to repeated infection out-of-doors.

The exact mechanism of the phenomenon has not been determined but the following points support the idea that this resistance is primarily a host reaction: the passage of soft pellets and diarrheic feces concomitantly with the loss of worms; the appearance of nodules in the skin after repeated cutaneous infection; the refractoriness to infection of rabbits which were originally susceptible to infection but have lost their worms; and the occurrence of self-cure in rabbits carrying infestations of greatly different magnitudes. The last two points would appear to furnish adequate proof that self-cure and resistance in rabbits is not related to mere parasitic overcrowding, since rabbits may be immune to infection although carrying no worms, and can discharge their worms although only few are present. The process appears then to be clearly analogus if not homologous to that observed by Stoll (1929) in sheep exposed to repeated infection with a stomach worm, *Haemonchus contortus*.

SUMMARY

Weekly increasing doses of the infective larvae of an intestinal nematode, *Trichostrongylus calcaratus*, were given to laboratory rabbits by mouth and by skin, and the resulting infections followed by frequent egg counts.

All of the rabbits were susceptible to infection at first as shown by increases in the egg counts roughly proportional to the number of larvae given, but after 6 to 8 weekly doses of larvae they became refractory to superimposed infection and finally discharged the worms which they did harbor. This self-cure manifested itself by sharp drops in the egg counts associated with passage of soft pellets and liquid feces containing adult worms.

The rabbits as tested by a later second series of infections were found to possess a high degree of acquired resistance. Those previously infected by mouth were resistant to later infection by mouth and by skin, and those infected initially by skin were also resistant to infection by either method.

In contrast to the sudden loss of infection from rabbits given repeated doses of larvae, the infection produced in a rabbit given a single dose of larvae showed a constant rate of loss over a period of 49 weeks, as determined by egg counts.

ACKNOWLEDGEMENT

The author is indebted to Dr. Norman R. Stoll for help in planning and starting the experiments and for assistance in the analysis and preparation of the results for publication.

REFERENCES CITED

Africa, C. M. 1931.—Studies on the host relations of *Nippostrongylus muris*, with special reference to age resistance and acquired immunity. Jour. Parasit., 18:1–13.

Chandler, A. C. 1932.—Susceptibility and resistance to helminthic infections. Jour. Parasit., 18:135–152.

Cort, W. W., Ackert, J. E., Augustine, D. L., and Payne, F. K. 1922.—Investigations on the control of hookworm disease. II. The description of an apparatus for isolating infective hookworm larvae from soil. Amer. Jour. Hyg., 2:1–16.

Fülleborn, F. 1926.—Ueber das Verhalten der Hakenwurmlarven bei der Infektion per os. Arch. Schiffs- Trop.-Hyg., 30:638–653.

Graham, G. L., Ackert, J. E., and Jones, R. W. 1932.—Studies on an acquired resistance of chickens to the nematode, *Ascaridia lineata* (Schneider). Amer. Jour. Hyg., 15:726–740.

Herrick, C. A. 1928.—A quantitative study of infections with *Ancylostoma caninum* in dogs. Amer. Jour. Hyg., 8:125–157.

McCoy, O. R. 1931a.—Immunity reactions of the dog against hookworm (*Ancylostoma caninum*) under conditions of repeated infection. Amer. Jour. Hyg., 14:268–303.

1931b.—Immunity of rats to reinfection with *Trichinella spiralis*. Amer. Jour. Hyg., 14:484–494.

Payne, F. K. 1923.—Investigations on the control of hookworm disease, XXXI. The relation of the physiological age of hookworm larvae to their ability to infect the human host. Amer. Jour. Hyg., 3:584–597.

Ransom, B. H. 1911.—Two new species of parasitic nematodes. Proceed. U. S. Nat. Museum, 41:363–369.

Sandground, J. H. 1928.—Some studies on susceptibility, resistance, and acquired immunity to infection with *Strongyloides stercoralis* (Nematoda) in dogs and cats. Amer. Jour. Hyg., 8:507–538.

Sarles, M. P. 1929.—The length of life and rate of loss of the dog hookworm, *Ancylostoma caninum*. Amer. Jour. Hyg., 10:667–682.

1930.—The occurrence of self-cure and protection in rabbits receiving repeated infections of *Trichostrongylus calcaratus* Ransom. Jour. Parasit., 17:114.

Schwartz, B., Alicata, J. E., and Lucker, J. T. 1931.—Resistance of rats to superinfection with a nematode, *Nippostrongylus muris*, and an apparently similar resistance of horses to superinfection with nematodes. Jour. Wash. Acad. Sc., 21:259–261.

Scott, J. A. 1928.—An experimental study of the development of *Ancylostoma caninum* in normal and abnormal hosts. Amer. Jour. Hyg., 8:158–204.

Stoll, N. R. 1929.—Studies with the strongyloid nematode, *Haemonchus contortus*. I. Acquired resistance of hosts under natural reinfection conditions out-of-doors. Amer. Jour. Hyg., 10:384–418.

1930.—On methods of counting nematode ova in sheep dung. Parasit., 22:116–136.

1932.—Note on re-infection under "natural" conditions with a gut nematode of the rabbit. Jour. Parasit., 19:54–60.

SOCIETY PROCEEDINGS

HELMINTHOLOGICAL SOCIETY OF WASHINGTON

One Hundred Forty-fourth to One Hundred Forty-eighth Meetings.

The one hundred forty-fourth meeting was held January 16, 1932.

The occurrence of *Moniezia benedeni* in a peccary by J. E. Alicata.—In November, 1931, the viscera from a peccary, *Pecari angulatus angulatus,* which had been killed in Texas, were sent to the Zoological Division. In the small intestine was found a tapeworm, apparently immature, which was identified as *Moniezia benedeni.* The tapeworm measured about 40 mm. in length by 8.5 mm. in maximum width. The worm was broken in the neck region and no head was found. The male and female genital organs were seemingly completely formed, but no gravid proglottids were present. This is apparently the first report of the occurrence of an anoplocephaline tapeworm from a peccary in the United States. There is only one other species of tapeworm, *Schizotaenia decrescens,* which has been reported from the collared and white-lipped peccaries of South America. From time to time tapeworms have been reported from domestic and wild swine. Kholodkovskiĭ (1894) was the first to find a cestode in the domestic pig, which is now known as *Thysanosoma giardi.* Undetermined anoplocephaline tapeworms from domestic swine have been reported by Stiles (1895), Oettle (1915), and Hall (1922). Bodkin and Cleare (1916), and Cameron (1930), have reported the finding of *Moniezia expansa* in the intestine of the pig. Maplestone and Southwell (1923), reported *Paramoniezia suis* from the wild pig which they called "Sus scrofula." Baylis (1927), reported *Paramoniezia phacochoeri* from the wart-hog *Phacochoerus aethiopicus,* and *Pseudanoplocephala crawfordi* from the wild boar *Sus cristatus.*

The following note was presented for Seymour Hadwen: In connection with a paper by R. T. Leiper (1931) on the Helminth Parasites of Deer, the following additional records are offered: *Fasciola magna* from *Odocoileus columbianus* from Texada Island, B. C.; *Echinococcus granulosus* from *Alces alces* at Le Pas, Manitoba, 1929; and *Cysticercus tenuicollis* from *Cervus elaphus* at Wainwright, Alberta, 1923.

G. Dikmans stated that the B. A. I. Helminthological Collection catalogue also contains a record of *Cysticercus tenuicollis* from the Newfoundland caribou, *Rangifer tarandus terrae-novae.*

G. Dikmans presented notes, as follows: (1) A case of aberrant parasitism. —Some nematodes collected from the intestine of a raccoon, *Procyon lotor,* from Gibson, La., were submitted to B. G. Chitwood for examination, who identified them as *Rhigonema infecta,* and stated that these nematodes are normally parasites of millipeds.

(2) *Ostertagia marshalli* from the mountain sheep, *Ovis canadensis; Ostertagia marshalli* and *O. occidentalis* from a deer, probably *Odocoileus hemionus hemionus,* both from the Yellowstone National Park.—The specimens of *O. marshalli* and *O. occidentalis* from the deer were collected by H. B. Raffensperger of the Zoological Division; those of *O. marshalli* from the bighorn sheep were collected by Hadleigh Marsh of the Montana Experiment Station. The B. A. I. Helminthological Collection contains specimens of this nematode collected from *Ovis canadensis* by Hall and Creech in 1925. *O. marshalli* and *O. occidentalis* were originally reported by the late Dr. Ransom as parasites of the domestic sheep from Montana. Inasmuch as there are no reports of the occurrence of these nematodes in domestic sheep in any other parts of the United States or of the world, it has been suspected that they were originally parasites of the wild ruminants found in that area. This report of their occurrence in wild ruminants lends

support to that idea and emphasizes again the necessity of securing accurate information as to the parasitic fauna of our wild ruminants.

(3) *Skrjabinema ovis*, reported in 1927 by B. Schwartz from goats in Nebraska has been found among some nematodes collected from a goat at Beltsville, Md. This goat was born, lived and died at Beltsville. This indicates that this nematode has become established in a new locality.

(4) The pocket gopher, *Thomomys fossor*, a new host of the nematode, *Hepaticola hepatica*.—The liver of a pocket gopher from the Medicine Bow Mountains, Wyoming, was submitted to the Zoological Division for examination on June 18, 1931. The liver showed on examination eggs similar to those of *Hepaticola hepatica*. A section of a nematode collected from this liver contained similar eggs.

W. E. Dove presented a discussion of his work at Charleston, S. C., on the breeding habits and control of sand-flies.

A note on the "albumin gland" of trematodes by E. W. Price.—In two recent papers (Studien über die Phylogenie der Trematoden IV and V) Sinitsin describes as the "albumin gland" a structure in connection with the female genital system of trematodes which he appears to regard as a new organ, or a structure which other writers have mistaken for the ootype. The writer has reexamined the material forming the basis for these papers and finds that the "albumin gland" is the well known Mehlis' gland or so-called "shell gland" and not a structure confused with the ootype, which is that portion of the uterus surrounded by Mehlis' gland.

Miss M. S. Skinker presented the following notes: (1) *Cysticercus cellulosae* in man.—A bottle with the contents labeled "Cysticercus from man," was sent to the Bureau of Animal Industry from Miles City, Montana. The collector is unknown. The specimens were determined as *Cysticercus cellulosae* on the basis of the hooks. If the information on the label is accurate, the specimens provide another definite record of this parasite in man.

(2) A new host for *Duthiersia fimbriata*.—Specimens of tapeworms collected from the iguana, *Cyclura stejnegeri*, from the West Indies by Dr. E. W. Price, Oct. 21, 1929, have been identified as *Duthiersia fimbriata*. According to available records, this is the first report of this parasite in the iguana. Several species of the lizards belonging to the genus Varanus have previously been reported as hosts.

G. Steiner discussed the ubiquity of nemas, with special reference to the distribution of Pathoaphelenchus.

The one hundred forty-fifth meeting was held February 20, 1932.

Stephanurus morai de Almeida, 1928, a synonym of *Stephanurus dentatus* Diesing, 1839, by J. E. Alicata.—De Almeida (1928), reported a new species of kidney worm from the pig which he called *Stephanurus morai*, and differentiated it from *Stephanurus dentatus* on the basis of the position of the vulva which he described as close to the anal opening. That the position of the vulva is close to the anal opening in *S. dentatus* is a well known fact. Unfortunately De Almeida based his determination on an erroneous statement made by Neveu-Lemaire (1912) that the vulva of *S. dentatus* is located in the middle of the body. In view of this, *Stephanurus morai* de Almeida, 1928, becomes a synonym of *Stephanurus dentatus* Diesing, 1839.

B. G. Chitwood presented for Miss Josephine Irey and himself a discussion of the nervous system of *Oesophagostomum dentatum*.

N. A. Cobb presented additional information in regard to a Tylenchus in lawn grass in South Africa, a form which is apparently closely related to *Tylenchus tritici*.

W. W. Cort discussed three types of hookworm disease in dogs, as follows: (1) Chronic type in natural and experimental infestations without symptomatology. (2) Acute type produced experimentally in repeated infestation. (3) Chronic severe hookworm disease.

G. Dikmans presented the following notes: (1) Ascaris infestation in the liver of a calf.—The specimen was forwarded to the Zoological Division by C. F. Payne, Inspector in Charge of Meat Inspection at National Stock Yards, Ill. The letter accompanying the specimen stated as follows: "The calf from which the specimen was taken was approximately eight weeks old and in rather poor condition. It is estimated that there were in quantity approximately one gallon of the parasites in the intestinal tract, which practically filled the lumen of the intestines and in many places distended the walls."

(2) On the distribution of anaplasmosis in the United States and the ticks experimentally determined as vectors of the disease.—Anaplasmosis was undoubtedly first noticed, although unrecognized as a separate disease entity, by Smith and Kilborne during their work on piroplasmosis or cattle tick fever. In 1910 Theiler, in South Africa, demonstrated that it was a disease separate and distinct from piroplasmosis. He gave the name *Anaplasma marginale* to the structures called "peripheral cocci" by Smith and Kilborne and which are found in the red blood cells of animals suffering from this disease. Since 1910 the disease has been reported from many parts of the world, occurring usually in association with or following attacks of piroplasmosis. Apparently Theiler's findings were not considered cause for alarm in the United States. It was assumed that the tick *Boophilus annulatus* was the carrier of both piroplasmosis and anaplasmosis and that the eradication of this tick would cause a disappearance of both diseases. For a long time, almost twenty years, it appeared as if this assumption was correct. From 1907, when the tick eradication campaign was started, the original tick quarantine line was gradually driven south and east as more and more territory was released from quarantine and we heard no more of tick fever in the released areas. In 1924 and subsequently reports began to drift in from areas released from quarantine and also from areas which had never been included in the original quarantine area, of the occurrence of a disease closely resembling tick fever. In 1927 the writer, then stationed at Jeanerette, La., was asked to make some preliminary investigations into the occurrence of anaplasmosis in Louisiana. A few trips to New Orleans soon demonstrated that this disease was quite a problem in that particular area. Information showing the occurrence of this disease in what are commonly known as tick-free areas, i. e., areas free from the cattle fever tick, *Boophilus annulatus,* was obtained. Since, as far as world reports are concerned, this disease is usually reported as occurring in association with tick fever in tick-infested areas and since we found it in a pure form in areas long since released from tick quarantine, it was considered necessary to determine beyond question that the disease we were dealing with was anaplasmosis, and our first experimental work was planned to settle that point. The next question was to determine how the animals acquired the disease. Since most reports on this disease incriminated some species of tick as a carrier, experiments were outlined to determine which of the ticks occurring in Louisiana, aside from the cattle fever tick, *Boophilus annulatus,* might act as carriers. We succeeded in transmitting the disease by means of the cattle tick in one case out of four trials. We failed to transmit the disease by means of larvae of *Amblyomma americanum* which were the progeny of adults engorged on anaplasmosis carriers. Unengorged adults of *Rhipicephalus sanguineus* and of *Ixodes scapularis* raised on rabbits, refused to attach. Since 1929 Dr. Rees has continued this work and to date he has shown that three species of ticks, *Rhipicephalus sanguineus, Dermacentor variabilis* and *Dermacentor andersoni* are able to transmit the disease.

The following notes were presented for C. W. Rees: (1) Additional confirmatory findings on the transmission of anaplasmosis by *Dermacentor variabilis.* —In previous publications the writer has reported four successful cases of transmission of anaplasmosis by *Dermacentor variabilis,* larvae engorged on clinical cases transmitting anaplasmosis as nymphs to susceptible bovines, and nymphs engorged on clinical cases transmitting it as adults to susceptible

bovines. A fifth experiment has just confirmed these findings. Nymphs of *D. variabilis* engorged on a clinical case of anaplasmosis and the subsequent generation of adults developed from them were placed on a susceptible bull on Dec. 21, 1931. Anaplasmosis developed in this bull on Jan. 19, 1932, the period of incubation being 29 days. All of the five experimental tests of larvae to nymph transmission and nymph to adult transmission were successful; on the other hand, all of four experimental tests of hereditary transmission from adults to larvae have been unsuccessful.

(2) The transmission of anaplasmosis by *Dermacentor andersoni.*—Anaplasmosis was successfully transmitted by *Dermacentor andersoni* in two cases. About 60 unengorged males and females of this tick were furnished for this experiment through the courtesy of R. R. Parker of the Rocky Mountain Spotted Fever Laboratory of the U. S. Public Health Service at Hamilton, Mont. About one-third of these were placed on a bull showing clinical anaplasmosis, about one-third on a heifer with clinical anaplasmosis, and about one-third on a bull which was a carrier of anaplasmosis. Two engorged females were recovered from the bull with clinical anaplasmosis, and two from the bull which was a carrier of anaplasmosis, and eggs from three of the four ticks hatched; partially engorged females recovered from the heifer failed to oviposit. Seed ticks from the eggs which hatched were placed on two susceptible bulls, but neither animal developed anaplasmosis by transmission from adult ticks to larval ticks. The larval ticks were then placed on two bulls, one a case of clinical anaplasmosis. Engorged larvae recovered from the clinical case of anaplasmosis molted and the nymphs of this generation were placed on two susceptible bulls on Jan. 4, 1932. One of these bulls developed anaplasmosis on Feb. 2 and the other on Feb. 3, 29 and 30 days after exposure to the ticks. This experiment demonstrates that *D. andersoni* will transmit anaplasmosis when larvae are engorged on clinical cases of anaplasmosis and the next generation of nymphs are engorged on susceptible animals. The addition of *D. andersoni* to the list of known tick carriers of anaplasmosis completes the case for ticks as possible carriers of anaplasmosis in the United States over the known range of distribution of anaplasmosis in this country. *D. variabilis,* previously convicted by the writer as a carrier, occurs practically throughout the United States except in the Rocky Mountain region. *D. andersoni* occurs throughout the Rocky Mountain region as well as on the West Coast. *Rhipicephalus sanguineus,* the third of the ticks incriminated by the writer, occurs in the South and in such northern cities as Chicago, Indianapolis and Philadelphia.

The one hundred forty-sixth meeting was held March 19, 1932.

The following notes were presented for C. W. Rees: (1) Transmission of anaplasmosis by adults of *Dermacentor andersoni.*—Anaplasmosis was successfully transmitted in two cases by means of nymphs of *Dermacentor andersoni* engorged on clinical cases of anaplasmosis and transmitting the disease as adult ticks to susceptible bulls. The incubation period was 31 days in one case and 32 days in the other. The successful transmission of anaplasmosis by this tick as a larva engorged on clinical cases and transmitting the disease as a nymph engorged on susceptible bulls, has already been reported by the writer. Two experiments in larva to nymph transmission, and two in nymph to adult transmission, have all been positive. The fact that all of the four tests made have been positive indicates that this tick would probably function very effectively as a carrier of anaplasmosis in nature, so far as transmission by the tick through one molt to the next stage in the tick life history is concerned. On the other hand, attempts to transmit anaplasmosis from adults engorged on clinical cases to larvae engorged on susceptible animals in the two experiments carried out up to date, have failed.

(2) Transmission of anaplasmosis by adults of *Rhipicephalus sanguineus.*—Anaplasmosis was successfully transmitted in two cases by means of nymphs of

Rhipicephalus sanguineus engorged on clinical cases of anaplasmosis and transmitting the disease as adult ticks to susceptible bulls. The incubation period was 31 days in both cases. The writer has previously reported the successful transmission of anaplasmosis by means of *R. sanguineus* engorged as larvae on a clinical case and transmitting the disease as nymphs to a susceptible bull. Here, as in the case of *Dermacentor andersoni,* hereditary transmission from adult to larva through the tick egg, has not been established.

In comment on these notes, M. C. Hall called attention to the fact that the work of Dr. Rees had established the transmission of anaplasmosis from larva to nymph, and from nymph to adult, for *R. sanguineus, D. andersoni,* and *D. variabilis* and had failed to establish it for hereditary transmission in the case of any of these ticks. Obviously three-hosts ticks can function as transmitters of disease in the stages preceding and following a molt, and yet be incapable of hereditary transmission. On the other hand, a one-host tick, such as *Boophilus annulatus,* is necessarily incapable of transmission other than hereditary transmission, since it leaves its host only as an adult.

Salt treatment for warble larvae in cattle by Seymour Hadwen.—Brine has been used for several generations by certain Canadian farmers as a larvacide applied to the backs of cattle to destroy warbles. The backs of the cattle are clipped for about 6 inches on either side of the backbone and saturated brine solution is brushed on the back once a week and allowed to dry. The application begins as early as October and in most instances is stopped during the month of February. Some of the farmers believe that this is efficacious and others are not sure whether it is. To test this treatment, a preliminary experiment was made by Mr. Henry, Research Fellow of Leather of the Ontario Research Foundation, by taking a freshly flayed piece of cattle hide, making a bag of this and filling the bag with brine and suspending it in a beaker of distilled water. He found that the salt diffuses through the entire thickness of the skin in from one-half to one hour, the diffusion being more marked in two to three hours. Dr. Hanes of the Ontario Research Foundation carried out some experiments on the oxygen consumption of individual larvae under various conditions of sodium chloride concentration, by means of Barcroft differential manometers. The rate of oxygen consumption of young and old larvae in contact with water or physiologic saline shows a very slow downward drift. The addition of 10 per cent sodium chloride causes the oxygen consumption of young larvae to fall away rapidly to zero in the course of about three hours, but in a single experiment on an old larva the rate of oxygen consumption remained approximately level for about 6 hours. There was also a difference in the change in volume of old and young larvae, as 10 per cent saline solution produced considerable shrinkage in young larvae in the course of the first 5 or 6 hours, but did not in old larvae. In field investigations it was found that the salt treatment does not injure the skin of the backs of cattle to any appreciable extent. Two or three days after the first application there is usually a very slight reddening, but this does not show in subsequent treatments and the washing gives the skin a clean, healthy appearance. Applications of salt were found to kill the grubs, but the older larvae were not so amenable to this treatment as the younger ones. The method deserves further investigation.

Sodium chloride for the destruction of hookworm larvae by Ronald G. Law. —Investigations with sodium chloride for the destruction of hookworm larvae gave promising results in a series of experiments carried out with silver foxes. Laboratory methods consisted of culturing infected feces with charcoal and recovering the larvae by the use of the Baermann isolation apparatus. The larvae were recovered in large quantities and counted by dilution, and subsequently planted in flower pots containing sterile soil. The larvae were covered with about an eighth of an inch of fine soil and left untreated for two days in order that they might establish themselves in their new environment. Controls were kept in all cases. The soil was then saturated to a depth of about one inch

with brine applied with a fine sprayer and left for two days, when it was returned to the Baermann apparatus. Negative results were obtained from the treated soil, while the controls showed numerous living larvae. The experiment was then carried to the breeding pens; three foxes which had tested negative by the Lane D. C. F. centrifuge for a period of one month were placed in pens known to be heavily infested with hookworm larvae. Two of these pens were treated with brine solution. Both foxes in the treated pens remained negative to hookworm for a period of two months, when the tests were discontinued. The fox in the untreated pen gave a positive test for hookworm eggs fourteen days after being placed in it. During the summer twenty-eight foxes marked for pelting were put in fifteen pens, and pilled with tetrachlorethylene, and the pens treated with brine solution eight days after the anthelmintic was given. These foxes were subsequently pelted in December and showed a remarkable freedom from hookworms.

A note on the genus Camallanus by B. G. Chitwood.—In reviewing the genus Camallanus the writer has examined the type specimens of the species described by MacCallum and finds that *Camallanus bungari* MacCallum, 1918, belongs to the genus Kalicephalus. A superficial examination of the literature of the genus Kalicephalus does not reveal a species with which it is clearly synonymous and the name is, therefore, changed to *Kalicephalus bungari* (MacCallum, 1918). Examination of the type specimen of *Camallanus cyathocephalus* MacCallum, 1918, shows it to be an immature specimen of *C. microcephalus* (Dujardin, 1845). *Camallanus scabrae* MacCallum, *C. troosti* MacCallum, *C chelydrae* MacCallum, *C. floridianae* MacCallum, *C. elegans* MacCallum, *C. testudinis* MacCallum, were also found to be synonymous with *C. microcephalus* (Dujardin, 1845). In addition, *C. seurati* Magath, *C. americanus* Magath, and *C. trispinosus* Leidy are all regarded as synonyms of *C. microcephalus* (Dujardin), since there appear to be no characters which will clearly separate these forms.

An additional record of *Diphyllobothrium latum* from bears in North America by Mary S. Skinker.—Specimens of cestodes from *Euarctos ursus americanus* from the region of Salt Lake City, Utah, were collected by L. A. Woodbury in August, 1931. These have been identified as *Diphyllobothrium latum,* and the record is of interest on account of the emphasis which has recently been placed on the distribution of these parasites in North America.

E. W. Price presented the following notes: (1) On the genus Phagicola Faust, 1920.—In connection with a review of the genera of trematodes, it has been necessary to determine the status of the genus Phagicola Faust, 1920. According to the original description of the type species, *Phagicola pithecophagicola,* no posterior oral appendage or gonotyls were noted and the apparent absence of these structures, which would clearly establish its position in the Heterophyidae, has caused some confusion. Witenberg (1929) in his revision of the Heterophyidae recognized as of generic rank the subgenus Parascocotyle Stunkard and Haviland, 1924, and included in it *P. pithecophagicola* (Faust). Later (1930) he stated that he had examined the type specimens of *Phagicola pithecophagicola* but was unable to add anything to the description of the species as given by Faust. He also suggested the possibility of both Phagicola and Parascocotyle being valid genera, but that this would depend upon a restudy of new material of Faust's species. Recently, through the courtesy of Doctor Faust, the writer has had an opportunity to restudy the type specimens of *P. pithecophagicola* and has been able to demonstrate clearly the presence of a posterior oral appendage, which is quite long and extends as far as the pharynx. Two gonotyls are present in the genital sinus, and a globular seminal receptacle lies median to the ovary. These characters clearly show that Phagicola and Parascocotyle are synonymous. The writer is of the opinion that sufficient differences exist between Ascocotyle Looss and Phagicola Faust to warrant the latter being considered as a distinct genus and not as a sub-

genus as is the opinion of Travassos (1930). To the genus Phagicola (=Parascocotyle) the following species are referred: *Phagicola pithecophagicola* Faust, *P. minuta* (Looss), *P. ascolonga* (Witenberg), *P. longa* (Ransom), *P. arnaldoi* Travassos, *P. italica* (Alessandrini), *P. piriforme* (Blanc and Hedin), *P. angrense* Travassos, *P. nana* (Ransom), *P. diminuta* (Stunkard and Haviland), and *P. angeloi* Travassos.

(2) The dog as a host for *Alaria arisaemoides* Augustine and Uribe, 1927.—Recently a portion of the anterior part of the duodenum of a dog to which was attached a number of trematodes was forwarded by Dr. B. T. Simms, Corvallis, Ore., to the Bureau of Animal Industry. These parasites have been identified as *Alaria arisaemoides* Augustine and Uribe, a species originally reported from a red fox, *Vulpes fulva*. This record is of interest since it involves a domestic carnivore and was found in a locality quite remote from the type locality (Massachusetts).

A preliminary note on the use of Fouadin as a treatment for *Dirofilaria immitis* in the dog by Paul C. Underwood and Willard H. Wright.—The successful application of Fouadin (Sodium-Antimony III bis-pyrocatechin-disulphonate of sodium) in the treatment of schistosomiasis in man by Khalil, lead the writers to try this drug against *Dirofilaria immitis*. However, before the treatment was undertaken, it was learned that Dr. F. G. Phillip of Shanghai, China, had employed the drug with good clinical results in this condition. Khalil recommends for man an initial dose of 1.5 c.c. of a 6.3 per cent solution of Fouadin, on the second day a dose of 3.5 c.c., on the third day a dose of 5 c.c. and an additional dose of 5 c.c. on alternate days up to the fifteenth day, with a total dose of 40 cc. Intramuscular injections are said to be followed by no reaction. The use in dogs of Fouadin in the above-mentioned doses resulted in marked local and systemic reactions culminating in death from the sixth to the eighth day after the start of the treatment. At the time of death, microfilariae had either disappeared from the peripheral circulation or were present only in markedly diminished numbers. In another animal treated with a reduced dose of Fouadin, it was necessary to discontinue the treatment on the eleventh day owing to the severe systemic reaction. In this animal microfilariae disappeared from the peripheral circulation on the tenth day and at the present writing, 113 days after the beginning of the treatment, the blood has remained negative to examinations for microfilariae. In those animals which succumbed to the treatment, live Dirofilaria were found in the heart.

W. W. Cort presented a discussion on the distribution of Ascaris, hookworm, Trichuris and Hymenolepis in Tennessee based on about 25,000 egg counts.

D. A. Shorb discussed strains of Hymenolepis in rats and mice.

Miss Josephine Irey presented a note on the excretory system of *Micropleura vivipara*.

The one hundred forty-seventh meeting was held April 16, 1932.

Uracanthus brevispinosus Diesing, 1861 by B. G. Chitwood.—In connection with a review of the types of obscure nematode genera, it has been found that Diesing (1861) renamed *Nematoideum passali* Leidy, 1852 and placed it in a new genus, the name being *Uracanthus brevispinosus* Diesing, 1861. Uracanthus Diesing is a homonym of Uracanthus Hope, 1835 (Coleop.) and therefore cannot be used. The specific name *brevispinosus* Diesing, 1861 is a synonym of *passali* Leidy, 1852. *Nematoideum passali* Leidy, 1852 and *Uracanthus brevispinosus* Diesing, 1861 are synonyms of *Chondronema passali* (Leidy, 1852) Christie and Chitwood, 1931. It appears that this genus should be placed in the Sphaerulariinae.

Protostrongylus macrotis from the black-tailed deer, *Odocoileus columbianus*, by G. Dikmans.—Some nematodes collected from the bronchi of a black-tailed deer by Hadleigh Marsh of Montana in February, 1932, have been identified as *Protostrongylus macrotis*. This nematode was described originally from the mule deer, *Odocoileus hemionus hemionus*.

Ostertagia odocoilei from the fourth stomach of a deer, *Odocoileus virginianus,* in Louisiana by G. Dikmans.—This nematode was described originally from this same host in Pennsylvania and this note is presented to record its presence in another locality.

Cooperia pectinata from the sable antelope, *Egocerus niger* by G. Dikmans.—This nematode which has so far only been reported as a parasite of cattle was found to be present in a collection of small trichostrongyles obtained by E. W. Price from a sable antelope from the National Zoological Park, Washington, D. C., in 1929.

Tapeworm cysts from the oesophagus of sheep by G. Dikmans.—D. R. Gillies, inspector in charge, Bureau of Animal Industry, New York, N. Y., recently sent to the Zoological Division four sections of oesophagi of sheep. Two oesophagi contained hard caseated nodules; one contained a cloudy nodule and one contained a clear transparent nodule. From the last nodule a cysticercus was removed which was identified by M. S. Skinker as *Cysticercus ovis.* The cloudy nodule was sectioned and on examination was found also to contain a cysticercus. In his paper on *Cysticercus ovis,* Ransom states that the parasites, *Cysticercus ovis,* have been found in a degenerate condition in the wall of the oesophagus; in a group of 99 sheep which were examined to determine the location of this parasite, it was found in the oesophagus in only two cases. Dr. Gillies informed us that no cysts were found in any other part of the bodies of these sheep.

D. A. Shorb presented a note on the transmission of Hymenolepis in rats.

A note on a new species of Pseudomermis by G. Steiner.—The material consisted of a single specimen, a female intersex with eggs in the uterus but also with well developed spicula, bursal muscles and three series of male copulatory papillae.

The successful transfer of *Aphelenchoides ritzema-bosi* from chrysanthemum to strawberry plants by G. Steiner.—As a result of the transfer *A. ritzema-bosi* produced the typical symptoms of the so-called dwarf-disease. A similar successful transfer of *A. olesistus* from ferns to strawberry plants had been made previously, which also resulted in dwarf symptoms. Given the close morphological similarity between *A. fragariae, A. olesistus* and *A. ritzema-bosi,* the present successful transfer to the strawberry plant and the production of identical symptoms seems to confirm their synonymy. Probably *A. subtenuis* should be included here also.

C. W. Stiles reported on parasitological observations made in Orange County, Fla. *Ancylostoma braziliense* occurs in the dog and cat in this area. A species of Moniliformis was found in the wood rat, *Neotoma* sp. Forty-nine per cent of the school children in this county are symptomatic hookworm cases, allowing 20 per cent for error in clinical diagnosis. Creeping eruption is found frequently.

Specimens of *Mononchus acutus* found to contain *Trichodorus obtusus, Tylenchus robustus* and *Xiphinema americana* by G. Thorne.—Specimens of *Mononchus acutus* from Arlington Farm, Va., were found to contain in the intestine bodies of *T. obtusus, T. robustus, X. americana,* setae and body fragments of an Oligochaete and a rotifer.

Apparent successful transfer of *Mononchus papillatus, M. muscorum* and *M. amphigonicus* from Utah to California by G. Thorne.—*M. papillatus, M. muscorum* and *M. amphigonicus* were apparently successfully transferred from their original habitat in Utah to the date gardens at Indio, Cal., for the purpose of learning if they may be of use in the control of *Caconema radicicola.*

The one hundred forty-eighth meeting was held May 21, 1932.

Occurrence of a nematode, *Cheilospirura spinosa* Cram, 1927, as a parasite of the sage grouse of Western United States by E. E. Wehr.—This nematode was reported for the first time from the ruffed grouse, *Bonasa umbellus,* in Michigan and has since been collected from birds in the states of Minnesota,

Wisconsin, New York, Massachusetts, New Jersey and Pennsylvania. The parasite has been reported also from the bob-white quail, *Colinus virginianus virginianus,* in Tennessee and Virginia. Recently, it has been collected from the gizzard of the sage grouse, *Centrocercus urophasianus,* in Wyoming and Montana. L. A. Woodbury is responsible for the collection of the material from Wyoming and the writer for the collection of the material from Montana.

On the differentiation of the infective third-stage larvae of the horse strongyles, *Strongylus equinus, S. edentatus* and *S. vulgaris* by E. E. Wehr.—Wetzel in a brief note presented before this society (J. Parasitology, v. xvii, June, 1931, p. 235) stated that it was possible to differentiate on the basis of morphology the ensheathed third-stage larvae of these three species of horse strongyles. The writer, not knowing that Dr. Wetzel was working on this problem, undertook during the summer of 1931 to find out if there were characters that were sufficiently constant to distinguish the third-stage larvae of the three above-mentioned species. Since Dr. Wetzel and the writer arrived at the same conclusions, this report must be considered in the nature of a confirmation of Dr. Wetzel's results.

Molineus patens (Dujardin, 1845) Skrjabin and Schultz, 1926, collected in the United States by M. S. Skinker.—A nematode collected by E. B. Cram from *Canis lestes* from the vicinity of Olympia, Wash., has been determined as *Molineus patens.* Other specimens collected by C. Walcott Curtice from a mink taken near McNeill, Miss., are also *Molineus patens.* This appears to be the first record of the collection of this parasite in North America, and *Canis lestes* is a host not previously reported. Sprehn (1928) reported having found nematodes belonging to this genus in the American mink, *Lutreola vison,* but the collection was evidently made in Germany and no record is given of the origin of the host.

Pathoaphelenchus parietinus (=*Aphelenchus ormerodis*) in the intestine of *Philomyscus dorsalis* (slug) by B. G. and M. B. Chitwood.—A single specimen of a slug, *Philomyscus dorsalis,* collected at Falls Church, Va., during the summer of 1929, was found to contain numerous immature aphelenchids. Recently during the examination of slugs and snails collected in Virginia, *Pathoaphelenchus parietinus* has been found in the intestine of the above-mentioned slug. This nematode is rather common in weeds in the vicinity of Glen Carlyn, Va., and presumably the nematodes have been taken in with the food. However, specimens of slugs which had been held under laboratory conditions for several days were found to contain the living nematode. Apparently this form is capable of surviving in the immature state in the digestive tract of slugs. This adds another possible mode of spread of plant parasitic nematodes.

The occurrence of *Ancylostoma caninum* in the raccoon, *Procyon lotor,* by B. G. Chitwood.—Recently a single female specimen of the common dog hookworm, *Ancylostoma caninum,* was collected from the small intestine of a raccoon, *Procyon lotor,* which died in the National Zoological Park, Washington, D. C. So far as the writer is aware this constitutes a new host record for this parasite.

The occurrence of *Platynosoma fastosum* in cats in Porto Rico by Wm. A. Hoffman.—*Platynosoma fastosum* Kossack, originally described from *Felis minuta* from the Königsberg Zoological Garden, has been recovered from the liver of domestic cats in the Malay States, Brazil and British Guiana. The reference of Baylis (A Manual of Helminthology) to the last locality probably pertains to the note of Bodkin and Cleare (Bull. Ent. Res., v. 7, 1916, p. 179). They stated that *Dicrocoelium* sp., near *D. lanceatum,* seemed extremely prevalent among cats of the colony. A number of trematodes, apparently of the above-mentioned species, were found by Prof. Garciá of the University of Porto Rico in the liver of a cat from Mayaguez, P. R. This species was reported by Dikmans (1927) and this constitutes a second record of the species for Porto Rico.

Experimental transmission of *Strongyloides ransomi* to a domestic rabbit by John T. Lucker.—Schwartz and Alicata (1930) reported that they failed to infect rabbits with *S. ransomi,* while *S. papillosus* was observed by them to develop to fertile maturity in these animals, as was first shown by Ransom in 1907. They concluded that *S. ransomi* is apparently not adapted to attain fertile maturity in rabbits. The following experiment has been performed by the writer. A domestic rabbit, in the feces of which no worm eggs were found on examination by the salt flotation method, was given orally 3800 infective larvae of *S. ransomi* obtained by culturing the feces of young pigs experimentally infected with this parasite. Five days later 30,000 additional larvae were given the animal by mouth. Nine days after the first feeding the initial appearance of eggs in the feces of the rabbit was noted. A fecal examination made subsequently indicated that the eggs agreed in structure and size with descriptions of eggs of *S. ransomi.* Sixteen days after the first feeding of larvae the animal was killed. The small intestine was flushed out with warm water and four mature *S. ransomi* were found in the washings. No immature forms were found in the intestine or lungs. It is evident from this experiment that fertile maturity was attained by an exceedingly small percentage of the excessively large number of infective larvae of *S. ransomi* which were administered to the rabbit. While the results of the experiment modify the conclusion reached by Schwartz and Alicata on the basis of their experimental feedings of *S. ransomi* to rabbits, their general premise that the rabbit is not a suitable host for this parasite is not invalidated.

The extraction of Oncocerca from the ligamentum nuchae by M. C. Hall.—The ligamentum nuchae from a calf in Porto Rico was found to be infected with Oncocerca and this specimen was put in a glass dish half full of water and about two teaspoonfuls of a 5 per cent solution of formaldehyde added. The next day a number of long fragments of the worms were found to have crawled out of the ligamentum and were preserved in good condition in the weak formaldehyde solution. This technic deserves further test.

Parasites in Porto Rico by M. C. Hall.—Tropical parasites are quite plentiful in man in Porto Rico, and the livestock show many of the parasites found in the temperate zones and several which are more or less restricted to the tropics. Profound anemia in man is a striking pathological condition. The presence of schistosomiasis in man and of liver flukes in cattle and goats establishes a condition which could be attacked to great advantage by a snail eradication campaign, especially under the conditions afforded by an island.

The transmission of piroplasmosis by means of a lancet by C. W. Rees.—Blood smears were taken from an animal which had shown a febrile reaction and the presence of *Piroplasma bigeminum* in the blood. The lancet used in securing the blood was dipped in a 5 per cent aqueous solution of lysol, wiped with absorbent cotton, and then used to make a blood smear from another animal. This second animal subsequently showed a febrile reaction and the presence of Piroplasma in the blood. These facts indicate that piroplasmosis may be transmitted by a lancet even when the lancet has been dipped in a 5 per cent solution of lysol and wiped with absorbent cotton. The presumption is that the perfunctory disinfection and cleaning of the lancet was not sufficient to sterilize or remove infective material. So far as can be judged from one experience, it appears that in handling piroplasmosis care should be taken to actually sterilize any instruments before they are used on susceptible animals.

The occurrence of more than one species of Piroplasma in the United States by E. Becker and C. W. Rees.—At least one species of Piroplasma, other than *P. bigeminum,* occurs in Louisiana. There is a possibility that this species is *P. argentinum* and Becker is inclined to think that it will be found to be this species. Rees does not concur but the writers agree that the variations are too great to permit the inclusion of both forms under *P. bigeminum.* Both writers recognize the necessity for additional investigation to settle the question.

Cooperia nicolli Baylis, 1929, a synonym of *Cooperia pectinata* Ransom, 1911 by G. Dikmans.—In 1929, Baylis described from cattle in Australia two nematodes which he named *Cooperia nicolli* n. sp. and *Cooperia fieldingi* n. sp. In reply to a written request dated Dec. 7, 1931, Dr. Baylis very kindly sent paratypes of these nematodes to the Zoological Division for a comparative study. A comparison of the paratypes of *C. nicolli* with *C. pectinata* shows that these nematodes are identical. Ransom in his original description of *C. pectinata* gives the length of the spicules as 240–280 μ and it was largely on the size of the spicules that Baylis based his opinion that the nematode described by him was a different species. A reexamination of the spicules of *C. pectinata* shows that they vary in length from 240–360 μ, whereas those of *C. nicolli* vary according to Baylis from 350–390 μ. The length of the muscular portions of the ovejectors including the sphincters is given by Ransom as 300 μ for *C. pectinata* and as 480–600 μ for *C. nicolli* by Baylis. An examination of about a dozen specimens of females of *C. pectinata* showed that the combined length of the muscular portions of the ovejectors including the sphincters varied from 328–670 μ in these specimens. *Cooperia nicolli* Baylis, 1929 is therefore a synonym of *Cooperia pectinata* Ransom, 1911.

The antelope, *Antilocapra americana,* a new host of the lungworm *Protostrongylus macrotis* by G. Dikmans.—Recently Hadleigh Marsh of the Veterinary Research Laboratory, Bozeman, Mont., sent to the Zoological Division some lungworms collected from an antelope in the Yellowstone National Park. These were determined as *Protostrongylus macrotis.* This nematode has previously been reported from the mule deer, *Odocoileus hemionus hemionus,* and the blacktailed deer, *Odocoileus columbianus.*

A trichostrongylid nematode erroneously placed in the genus Pelodera by G. Dikmans.—In a short note entitled "Mastdarmvorfall bei Laubfröschen und Nematoden" (Blätter fur Aquarien u. Terrarienkunde, v. 39 (23), Dec. 15, 1928), the author, Kurt Deckert, mentioned the occurrence of a nematode in the tree frog, *Hyla* sp. He stated that the nematodes probably belong in the genus Pelodera, formerly Rhabditis, but the figure accompanying the article very plainly shows that the nematode is not a member of that genus but is a trichostrongylid. Judging from the figure, the nematode belongs in the family Trichostrongylidae and on the basis of the host one might hazard an opinion that it belongs in the genus Oswaldocruzia, but this point cannot be determined with certainty from the evidence presented. The editor also states that through a regrettable oversight the figure representing this trichostrongyle was used in a previous number (21) of this publication to illustrate another article and it was there labelled *"Filaria candazei* Fraipont."

Artificial infestation of the English sparrow, *Passer domesticus domesticus,* with the gapeworm, *Syngamus trachea,* by Eugenia Cuvillier.—The worm cultures were obtained from infested Manchurian pheasants and were fed to two English sparrows. The first attempt was a failure, but on repeated feeding, one of the sparrows showed an infestation; post mortem it contained a single pair of adult worms. So far as can be ascertained from the literature, this constitutes a new host for the gapeworm, *Syngamus trachea.*

New records of nematodes of birds by E. B. Cram.—*Porrocaecum ensicaudatum* from intestine of the starling, *Sturnus vulgaris vulgaris,* at Quebec, Canada, collected by Alex D. Baker; and from intestine of the robin, *Turdus migratorius migratorius,* in New Jersey, collected by F. R. Beaudette. *Capillaria meleagris-gallopavo* from intestine of the ring-necked pheasant, *Phasianus colchicus torquatus,* in New Jersey, collected by J. J. Black. *Capillaria contorta* from oesophagus (dilated and undilated) of the turkey, *Meleagris gallopavo;* experimental development at Beltsville, Md., the adult nematodes being collected 72 days after the turkey had been fed a culture of the capillarid eggs, the eggs having been collected from the crop of a bob-white quail, *Colinus virginianus virginianus,* 80 days previous to their being fed to the turkey. Similar feedings

to two chickens gave negative results. *Capillaria retusa* from intestine of the turkey, *Meleagris gallopavo;* experimental development at Beltsville, Md., in two turkeys, by the feeding of capillarid eggs which had been found in droppings from turkeys located at the U. S. Soldiers Home, Washington, D. C. *Cosmocephalus obvelatus* from oesophagus of pelican, *Pelecanus* sp., at the National Zoological Park, Washington, D. C., collected by E. W. Price. *Chevreuxia revoluta* from under the corneus lining of the gizzard of the avocet, *Recurvirostra americana,* at Salt Lake City, Utah, collected by L. A. Woodbury. The names of the birds here recorded are in accordance with the new A. O. U. Check List of North American Birds.

An ascarid in the abomasum of a sheep by John S. Andrews.—On January 5, 1932, an immature ascarid was found in the abomasum of a lamb, killed at the abattoir of the Bureau of Animal Industry at Beltsville, Md. Specimens of this nematode have been recovered also on three separate occasions within the last month from the small intestines of sheep killed at a local abattoir. These nematodes have been reported from sheep on various occasions from different countries, mostly from the small intestine and from the bile ducts. Morphologically these ascarids are *Ascaris lumbricoides.*

N. A. Cobb discussed the marine nema, *Metoncholaimus pristiurus,* (Zur Strassen) as a subject for class room work in zoology, emphasizing the many advantages this form offers, i. e., convenient size, transparence, fascinating anatomy; that it can be studied while living even far distant from the ocean; that it is well adapted for transportation and may be kept for months in an ice box; and that it can be supplied from the Marine Biological Laboratory, Woods Hole, Mass., at almost any time.

Dr. Cobb referred also to a publication by Kaburaki and Imamura on a new Mermithid parasitic in the rice borer. The form involved is morphologically related to *Mermis subnigrescens.*

Dr. Cobb also spoke of having received from Prof. Gates of Rangoon, Burma, a very outstanding collection of nemas parasitic in tropical earthworms. Numerous new species and genera are represented with structures not yet known in other nemas.

W. H. Wright, Secretary.

AMERICAN SOCIETY OF PARASITOLOGISTS, ANNOUNCEMENTS

Preliminary Announcement of the 8th Annual Meeting of the American Society of Parasitologists, at Atlantic City, N. J., Dec. 28–30, 1932.

The council of the American Society of Parasitologists on April 25th approved Atlantic City as the place of the 8th annual meeting of the Society, to be held in conjunction with the American Association for the Advancement of Science. Due to Christmas Day falling on Sunday, the official opening of the A.A.A.S. has been set for Tuesday evening, rather than Monday as is usual, and in consequence the Parasitologists' program will begin on Wednesday morning, December 28th. Society headquarters will be at the Chelsea Hotel.

Besides the regular sessions devoted to the presentation of contributed scientific papers, especial attention is called to the opportunity to present papers wholly or in part by demonstrations. Authors utilizing charts and graphs in connection with oral reports are invited to so prepare them that they may be advantageously placed on display at the demonstration period as well.

The Atlantic City meetings will be under the presidency of Maurice Crowther Hall, whose presidential address will be a feature of the program. In view of the success attending the first dinner of the Society at New Orleans last year, the Second Annual Dinner of the Society will be held on Wednesday evening. Dr. Hall will preside. It is planned to have the annual business meeting of the Society follow the dinner.

Members will be interested to learn that in addition to the abstracts of papers to be presented, the December issue of the Journal of Parasitology will contain the program sequence.

NORMAN R. STOLL, Secretary.

ANNOUNCEMENT OF THE TRANSFER OF THE JOURNAL OF PARASITOLOGY TO THE AMERICAN SOCIETY OF PARASITOLOGISTS

Dr. Henry B. Ward established the Journal of Parasitology in September, 1914, and with the help of a Board of Editors representing parasitology in the United States has carried it through eighteen volumes. Throughout this period it has remained the only American journal devoted entirely to the subject of parasitology. This journal received immediate recognition both in this country and abroad, and took its place as one of the world's important publications devoted to the subject of parasitology. During this period Dr. Ward as managing editor and owner carried the editorial and financial responsibility.

The first society relationship established by the Journal of Parasitology was the publication, beginning with Volume 1, No. 2 (December 1914), of the Proceedings of the Helminthological Society of Washington.

By 1925 the subject of parasitology had developed to such an extent in the United States that the need for an organization of the workers in this field found expression in the development of the American Society of Parasitologists. Dr. Ward was the first president of this Society and then served for four years on its Council. Early in the Society's history an arrangement was worked out for an unofficial relationship with the Journal, which took the form of the printing in the Journal of Parasitology of the abstracts of papers presented at the annual meeting. This and other forms of cooperation led to the development of the proposal to work out a plan by which the Journal of Parasitology could be made the official organ of the American Society of Parasitologists. Therefore at a meeting of the Council of the Society held Dec. 17, 1927, a committee was appointed for the purpose of bringing about this union. In 1927 the Society was circularized on the subject of making the Journal of Parasitology its official organ, and a very favorable response was received, 83 per cent of the 281 sending in answers voting in favor of the proposition.

The committee on making the Journal of Parasitology the official organ of the American Society of Parasitologists continued its work but for one reason or another no final agreement was reached. During this period the strength of the Society greatly increased, its membership roll reaching 550. The sentiment favorable to an official publication also increased and at the Council meeting held Dec. 28, 1931, at New Orleans, a new committee on publication was appointed with instructions to take all possible steps to obtain an official journal of the Society.

This committee has been able to bring the negotiations with Dr. Ward to a satisfactory conclusion. At a committee and Council meeting held in Washington, April 25, 1932, Dr. Ward definitely offered to give the Journal of Parasitology to the American Society of Parasitologists following the publication of the last number of Volume 18, provided a vote of the Society indicated that the membership of the organization was willing to support the Journal. This vote was taken in May and of 315 members returning the post cards by May 31st, 284, or 90.2 per cent. voted in favor of the proposition and agreed to continue as members under the new conditions. On receipt of information as to the favorable vote, Dr. Ward formally offered the Journal as a gift to the Society and it was accepted by a unanimous vote of the Council. The Council, on the same occasion, then formally appointed as an editorial committee for a period of five years, W. W. Cort, Chairman, Robert Hegner, and Francis M. Root. It was deemed wise to delegate the main responsibility for editing and managing the Journal to a committee all resident in the same place. In addition a revolving

Editorial Board representative of various phases of parasitology and of different parts of the country was appointed as follows:

Entomology: F. C. Bishopp, U. S. Bureau of Entomology, 4 years; William A. Riley, University of Minnesota, 2 years.

Protozoology: Charles F. Craig, Tulane University, 1 year; Charles A. Kofoid, University of California, 2 years; William H. Taliaferro, University of Chicago, 3 years; David H. Wenrich, University of Pennsylvania, 4 years.

Helminthology: James E. Ackert, Kansas State College, 3 years: Asa C. Chandler, Rice Institute, 2 years; Maurice C. Hall, U. S. Bureau of Animal Industry, 1 year; George R. LaRue, University of Michigan, 4 years; Richard P. Strong, Harvard University, 3 years; Henry B. Ward, University of Illinois, 1 year.

The gift by Dr. Ward of the Journal of Parasitology to the American Society of Parasitologists gives this Society as its official organ a successful journal which has run through eighteen volumes, and which has a list of subscribers outside of Society members of over 400. The editorial committee has given the contract for printing to the Science Press of Lancaster, Pa, an organization devoted primarily to the printing of scientific journals. The Society owes a debt of gratitude to Dr. Henry B. Ward for his generous gift, which can be best repaid by making the Journal of Parasitology, which he founded and into which he put such effort, an increasingly important factor in the development of parasitology in the United States.

The Journal under Society auspices starts out with every chance of success.

Committee on Publication:

BENJAMIN SCHWARTZ, *Chairman*
H. E. EWING
ROBERT HEGNER
NORMAN R. STOLL, *ex officio*